TRANSGENIC PLANTS WITH IMPROVED PHENOTYPES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of prior application No. 10/310,154 filed December 4, 2002, which application claims priority under 35 U.S.C. §119(e) of U.S. Provisional Application No. 60/337,358 filed December 4, 2001, the disclosure of which application is incorporated herein by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

Two copies of the sequence listing (Copy 1 and Copy 2) and a computer readable form (CRF) of the sequence listing, all on CD-ROMs, each containing the file named Pa_00613.rpt, which is 84,936,704 bytes (measured in MS-WINDOWS) and was created on November 3, 2003, are herein incorporated by reference.

INCORPORATION OF TABLES

Two copies of Tables 1-3 on CD-ROMs, each containing the file named pa_00613.txt, which is 3,008,512 bytes (measured in MS-WINDOWS) and was created on November 3, 2003, are herein incorporated by reference.

FIELD OF THE INVENTION

Disclosed herein are seeds from transgenic plants, wherein the genome of said seed comprises recombinant polynucleotides, the expression of which results in the production of transgenic plants with enhanced phenotypes.

BACKGROUND OF THE INVENTION

Transgenic plants with improved agronomic traits such as yield, pest resistance, herbicide tolerance, improved seed compositions, and the like are desired by both farmers and consumers. Although considerable efforts in plant breeding have provided significant gains in desired phenotypes, the ability to introduce specific DNA into plant genomes provides further opportunities for generation of plants with improved and/or unique phenotypes. The ability to develop transgenic plants with improved traits depends in part on the identification of genes that

are useful in recombinant DNA constructs for production of transformed plants with improved properties.

SUMMARY OF THE INVENTION

The present invention is directed to seed from a transgenic plant line, wherein said seed comprises in its genome a recombinant polynucleotide providing for expression or suppression of a polypeptide provided herein. Of particular interest is seed from a transgenic plant line, wherein said seed may be grown to produce plants having increased yield as compared to the yield of a control plant. Increased yield may be characterized as plant yield increase under non-stress conditions, or by plant yield increase under one or more environmental stress conditions. The invention also provides transgenic seed for plant lines having other enhanced phenotypes, such as enhanced plant morphology, plant physiology or seed component phenotype as compared to a corresponding phenotype of a control plant line. Of particular interest in the present invention is seed from transgenic crop plants, preferably maize (corn – *Zea mays*) or soybean (soy – *Glycine max*) plants. Other plants of interest in the present invention for production of transgenic seed that can be grown to provide plants having enhanced properties include, without limitation, cotton, canola, wheat, sunflower, sorghum, alfalfa, barley, millet, rice, tobacco, fruit and vegetable crops, and turfgrass.

In one aspect, this invention relates to the generation of transgenic plants by transformation with recombinant polynucleotides, and the identification of transgenic plants comprising such recombinant polynucleotides and having enhanced phenotypes. Of particular interest are transgenic plants that exhibit an improvement in a plant trait that is a component of yield. This aspect of the invention employs recombinant polynucleotides for expression of polypeptides that are useful for imparting desired traits to the transformed plants and recombinant polynucleotides for expression of homologs of such polypeptides as described herein. Exemplary polynucleotides which encode polypeptides of interest in the present invention are provided as SEQ ID NO:1 through SEQ ID NO:339. Sequences of the polypeptides of interest are provided as SEQ ID NO:340 through SEQ ID NO:678, and sequences of exemplary homolog polypeptides are provided as SEQ ID NO:679 through SEQ ID NO:24149. Tables 1-3 identifying the sequences of the present invention and their homologs are provided on the CD-ROM filed herewith.

Also of interest are recombinant polynucleotides that provide for suppression of expression of a target gene in a transgenic plant host using gene suppression methods, such as antisense or RNAi. Any of the polynucleotides provided herein as SEQ ID NO:1 through SEQ ID NO:339 may be used in such recombinant polynucleotides for gene suppression. Of particular interest are recombinant polynucleotides for gene suppression in maize, wherein said polynucleotide targets gene suppression of the corn aquaportin RS81 protein SEQ ID NO:8 or the retinoblastoma-related protein 1 provided as SEQ ID NO:70.

Thus, the present invention also comprises recombinant polynucleotides. Recombinant polynucleotides exemplified herein comprise a promoter functional in a plant cell operably joined to a DNA segment comprising encoding sequence for a polypeptide provided herein, or a homolog thereof. Such molecules are useful for production of transgenic plants having at least one improved property as the result of expression of a polypeptide of this invention or suppression of expression of a polypeptide described herein.

Also considered in the present invention is a method of producing a plant having an improved property, wherein the method comprises transforming a plant with a recombinant polynucleotide providing for expression or suppression of a polypeptide provided herein, and growing said transformed plant. In one aspect, the recombinant polynucleotide comprises a promoter functional in a plant cell operably joined to a DNA segment comprising encoding sequence for a polypeptide provided herein. The polynucleotide may be oriented with respect to the promoter to provide for transcription of sense or antisense RNA, or a combination of sense and antisense RNA, such as for use in RNAi methods of gene suppression. Of particular interest are uses of such methods to generate transgenic crop plants having increased yield.

Another aspect of the invention provides fragments of the polynucleotides of the present invention for use, for example as probes or molecular markers. Such fragments comprise at least 15 consecutive nucleotides in a sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:339 and complements thereof. Polynucleotide fragments of the present invention are useful as primers for PCR amplification and in hybridization assays such as transcription profiling assays, marker assays, or crop identity assays, including, for example, high throughput assays where the oligonucleotides are present in high density on a substrate, such as for example in microarrays.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to seed from a transgenic plant, wherein the genome of said seed comprises an exogenous polynucleotide comprising a functional portion of an encoding region for a polypeptide provided herein, and wherein plants grown from said seed exhibit an enhanced phenotype as compared to the phenotype of a control plant. Of particular interest are plants wherein the enhanced phenotype is increased yield. Exogenous polynucleotides of the present invention include recombinant polynucleotides providing for expression of mRNA encoding a polypeptide, and recombinant polynucleotides providing for expression of mRNA complementary to at least a portion of an mRNA native to the target plant for use in gene suppression.

As used herein, a "transgenic plant" is one whose genome has been altered by the incorporation of exogenous genetic material, e.g. by transformation as described herein. The term "transgenic plant" is used to refer to the plant produced from an original transformation event, or progeny from later generations or crosses of a plant so transformed, so long as the progeny contains the exogenous genetic material in its genome. By "exogenous" is meant that a nucleic acid molecule, for example, a recombinant polynucleotide, originates from outside the plant into which it is introduced. An exogenous nucleic acid molecule may comprise naturally or non-naturally occurring polynucleotides, and may be derived from any organism, including the same or a different plant species than that into which it is introduced.

"Recombinant polynucleotide" refers in the present invention to a polynucleotide having a genetically engineered modification introduced through manipulation via mutagenesis, restriction enzymes, and the like. Recombinant polynucleotides may comprise DNA segments obtained from different sources, or DNA segments obtained from the same source, but which have been manipulated to join DNA segments which do not naturally exist in the joined form. A recombinant polynucleotide may exist outside of the cell, for example as a PCR fragment, or integrated into a genome, such as a plant genome.

As used herein, a "functional portion" of an encoding region for a polypeptide provided herein is a sufficient portion of the encoding region to provide the desired activity. Where expression of protein is desired, a functional portion will generally comprise the entire coding region for the polypeptide, although certain deletions, truncations, rearrangements and the like of the polypeptide may also maintain, or in some cases improve, the desired activity. One skilled in

the art is aware of methods to screen for such desired modifications and such polypeptides are considered within the scope of the present invention. Where gene suppression methods are employed, smaller portions of the encoding region may be used to produce the desired effect.

"Enhanced phenotype" as used herein refers to a measurable improvement in a crop trait including, but not limited to, yield increase, including increased yield under non-stress conditions and increased yield under environmental stress conditions. Stress conditions may include, for example, drought, shade, fungal disease, viral disease, bacterial disease, insect infestation, nematode infestation, cold temperature exposure, heat exposure, osmotic stress, reduced nitrogen nutrient availability, reduced phosphorus nutrient availability and high plant density. Many agronomic traits can affect "yield", including without limitation, plant height, pod number, pod position on the plant, number of internodes, incidence of pod shatter, grain size, efficiency of nodulation and nitrogen fixation, efficiency of nutrient assimilation, resistance to biotic and abiotic stress, carbon assimilation, plant architecture, resistance to lodging, percent seed germination, seedling vigor, and juvenile traits. Other traits that can affect yield include, efficiency of germination (including germination in stressed conditions), growth rate (including growth rate in stressed conditions), ear number, seed number per ear, seed size, composition of seed (starch, oil, protein) and characteristics of seed fill.

Also of interest is the generation of transgenic plants that demonstrate enhanced phenotypic properties that may or may not confer an increase in overall plant yield. Such properties include enhanced plant morphology, plant physiology or enhanced components of the mature seed harvested from the transgenic plant. Of particular interest are enhancements in seed oil, tocopherol, protein and starch components, including increases in the quantity of any of these components, alterations in the ratios of any of these components, or production of new types of these components that do not exist in the seed from control plants. By way of example, increases in total tocopherol content are desirable, as are increases in the relative percentage of a-tocopherol produced by plants.

A "control plant" as used in the present invention is a plant used to compare against a transgenic plant grown from transgenic seed provided herein, to identify an enhanced phenotype in said transgenic plant. A suitable control plant may be a non-transgenic plant of the parental line used to generate a transgenic plant herein. A control plant may in some cases be a transgenic plant line that comprises an empty vector or marker gene, but does not contain the

recombinant polynucleotide of the present invention that is expressed in the transgenic plant being evaluated. In general, a control plant is a plant of the same line or variety as the transgenic plant being tested.

"Increased yield" of a transgenic plant of the present invention may be evidenced and measured in a number of ways, including test weight, seed number per plant, seed weight, seed number per unit area (i.e. seeds, or weight of seeds, per acre), bushels per acre, tonnes per acre, tons per acre, kilo per hectare. For example, maize yield may be measured as production of shelled corn kernels per unit of production area, e.g. in bushels per acre or metric tons per hectare, often reported on a moisture adjusted basis, e.g. at 15.5 % moisture. Increased yield may result from improved utilization of key biochemical compounds, such as nitrogen, phosphorous and carbohydrate, or from improved responses to environmental stresses, such as cold, heat, drought, salt, and attack by pests or pathogens. Polynucleotides of the present invention may also be used to provide plants having improved growth and development, and ultimately increased yield, as the result of modified expression of plant growth regulators or modification of cell cycle or photosynthesis pathways.

"Expression" as used herein refers to transcription of DNA to produce RNA. The resulting RNA may be without limitation mRNA encoding a protein, antisense RNA that is complementary to an mRNA encoding a protein, or an RNA transcript comprising a combination of sense and antisense gene regions, such as for use in RNAi technology. Expression as used herein may also refer to production of encoded protein from mRNA.

"Gene suppression" is used herein to refer to reduction or suppression of expression of a target protein in a host cell as the result of transcription of a recombinant polynucleotide provided herein, wherein the polynucleotide is oriented with respect to a promoter to provide for production of RNA having a gene silencing effect, such as antisense RNA or interfering RNA (RNAi).

Transgenic Plants and Seed

Transgenic plant seed provided by this invention may be grown to generate transgenic plants having an enhanced phenotype as compared to an appropriate control line. Such seed is obtained by screening transformed plants for enhanced phenotypes resulting from the introduction of a recombinant polynucleotide into the genomic DNA of tissue from a parental

line. The recombinant polynucleotide is introduced into the genome to produce transgenic cells that can be cultured into transgenic plants having an enhanced phenotype as compared to the parental line or other appropriate control. Such transgenic cells are cultured into transgenic plants that produce progeny transgenic seed. Preferably, multiple transgenic plants (events) comprising the recombinant polynucleotides are evaluated, e.g. from 2 to 20 or more transgenic events, to identify a desired enhanced phenotype. Although the design of a recombinant polynucleotide is based on a rational expectation of a phenotypic modification, the present invention also contemplates that unexpected, yet desired enhanced phenotypes may be obtained.

Transgenic plants grown from transgenic seed provided herein demonstrate improved phenotypes that contribute to increased yield or other increased plant value, including, for example, improved seed quality. Of particular interest are plants having altered cell division, enhanced plant growth and development, stress tolerance, including tolerance to abiotic and biotic stress, altered seed or flower development, improved light response, and enhanced carbon and/or nitrogen metabolism, transport or utilization properties.

Yield enhancements by modification of cell division may be obtained, for example, by expression of cyclins, cytokinins, cyclin activating kinases or E2F or suppression of retinoblastoma 1.

Plant growth and development enhancements may be obtained, for example, by modification of expression of F box proteins or heterotrimeric G proteins, by modification of steroid biosynthesis and signaling or plant architecture, and by modification of activity of key plant development components, such as elongation factors, growth regulators and various transcription factors.

Stress tolerance enhancements may be obtained, for example by modification of expression of genes involved in heat tolerance, such as HSP90 and HSF genes; genes involved in cold tolerance, such as cold induced genes including SEQ ID NO:147 and SEQ ID NO:168 through SEQ ID NO:176, and fatty acid desaturase genes; genes associated with improved water use efficiency, such as *Arabidopsis* transcription factor G975 and crop homologs of G975; genes involved in disease resistance, including yeast superkiller (SKI) genes and plant superkiller homologs, or pest tolerance; genes associated with oxidative stress tolerance, such as provided as SEQ ID NO:241 through SEQ ID NO:272; genes associated with phospholipid signaling,

jasmonate biosynthesis or flavonoid biosynthesis, or genes encoding phosphoinositide binding proteins, such as SEQ ID NO:331 through SEQ ID NO:335.

Seed development enhancements may be obtained, for example by modification of nitrate transport, modification of nucellin like proteins related to *dsc*1 and modification of expression of SET domain proteins, such as for alteration of endosperm or embryo size, or production of apomixis.

Light response enhancements may be obtained, for example by modification of expression of phytochrome or genes involved in phytochrome regulation or signal transduction genes such as provided as SEQ ID NO:23 through SEQ ID NO:31, SEQ ID NO:53 through SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:98, SEQ ID NO:11 through SEQ ID NO:113, SEQ ID NO:207, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:230, SEQ ID NO:240, SEQ ID NO:277 and SEQ ID NO:311 through SEQ ID NO:315.

Flower development enhancements may be obtained, for example by modification of expression of genes related to flowering time such as provided herein as SEQ ID NO:40 through SEQ ID NO:43 and SEQ ID NO:326 through SEQ ID NO:328 and corn ear development, such as provided herein as SEQ ID NO:17 and SEQ ID NO:213.

Nitrogen utilization enhancements, including improved seed or grain quality, may be obtained, for example by modification of expression of genes involved in nitrogen assimilation, metabolism or transport.

Plant enhancements by alteration of source and/or sink properties are also considered in the present invention and may be obtained, for example, by improvements to sucrose production and/or transport, such as by expression of SEQ ID NO:279 through SEQ ID NO:283 and SEQ ID NO:298 through SEQ ID NO:308, or by modification of carbon partitioning.

Also of interest are plants having increased yield as the result of expression of genes, that are transcriptionally regulated in a manner that correlates with high yield, or by expression of homologs of such genes.

Polypeptides useful for generation of transgenic plants having enhanced properties are described in Table 4 below and provided herein as SEQ ID NO:340 through SEQ ID NO:678. Column headings in Table 4 refer to the following information:

"PEP SEQ ID NO" refers to a particular amino acid sequence in the Sequence Listing

"PHE ID" refers to an arbitrary number used to identify a particular recombinant polynucleotide corresponding to the translated protein encoded by the polynucleotide.

"NUC SEQ ID NO" refers to a particular nucleic acid sequence in the Sequence Listing which defines a polynucleotide used in a recombinant polynucleotide of this invention.

"GENE NAME" refers to a common name for the recombinant polynucleotide.

"GENE EFFECT" refers to the effect of the expressed polypeptide in providing yield improvement or other enhanced property

"CODING SEQUENCE" referS to peptide coding segments of the polynucleotide.

"SPECIES" refers to the organism from which the polynucleotide DNA was derived.

TABLE 4

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
340	PHE0000001	1	maize cellulose synthase (eskimo 2)	Cold tolerance	113-3061	Zea mays
341	PHE0000006	2	Arabidopsis RAV2/G9	Root mass	81-1136	Arabidopsis thaliana
342	PHE0000007	3	rice G9-like 1	Root mass	336-1430	Oryza sativa
343	PHE0000008	4	rice G9-like 2	Root mass	572-1522	Oryza sativa
344	PHE0000010	5	rice G975	Water use efficiency	201-283,516-1161	Oryza sativa
345	PHE0000278	6	corn G975	Water use efficiency	41-679	Zea mays
346	PHE0000011	7	corn Glossy15	Water use efficiency	385-1722	Zea mays
347	PHE0000012	8	corn aquaporin RS81	Root mass	1-747	Zea mays
348	PHE0000014	9	rice cycD2	Cell division	13-324,623-709,813-911,1003-1204,1314-1438,1529-1774	Oryza sativa
349	PHE0000215	10	invW	Sucrose production/transport	1108-1489,1813-2684,6105-6266,6417-6658,	Oryza sativa
350	PHE0000015	11	rice GCR1	Cell division	312-500,1123-1154,1384-1553,2048-2163,2724-2825,2946-3002,3331-3474,3930-4000,4118-4223	Oryza sativa
351	PHE0000016	12	corn Knotted1	Cell division	181-1257	Zea mays
352	PHE0000018	13	corn AAA-ATPase 2	Plastid division	104-2533	Zea mays
353	PHE0000019	14	rice AOX1b (alternative oxidase)	Cold tolerance	4531-4851,5011-5139,6072-6560,6663-6722	Oryza sativa

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
354	PHE0000020	15	Emericella nidulans alxA	Cold tolerance	2189-2442,2492-2783,2843-3352	Emericella nidulans
355	PHE0000022	16	corn AAP6-like	Nitrogen transport	96-1547	Zea mays
356	PHE0000024	17	corn unknown protein	Flower development	441-2390	Zea mays
357	PHE0000025	18	corn GRF1-like protein	Plant growth and development	55-1470	Zea mays
358	PHE0000026	19	rice GRF1	Plant growth and development	193-1380	Oryza sativa
359	PHE0000227	20	soy omega-3 fatty acid desaturase	Cold tolerance	138-1496	Glycine max
360	PHE0000258	21	AtFAD7	Cold tolerance	132-1472	Arabidopsis thaliana
361	PHE0000259	22	AtFAD8	Cold tolerance	61-1368	Arabidopsis thaliana
362	PHE0000049	23	rice phyA with corn phyC intron 1	Light response	4626-6690,6913-7729,8011-8307,8410-8617	Oryza sativa
363	PHE0000027	24	sorghum phyA with corn phyC intron 1	Light response	238-3633	Sorghum bicolor
364	PHE0000028	25	rice phyB with corn phyC intron 1	Light response	67-3582	Oryza sativa
365	PHE0000029	26	sorghum phyB with corn phyC intron 1	Light response	429-2640,3333-4140,5819-6112,7491-7713	Sorghum bicolor
366	PHE0000030	27	rice phyC with corn phyC intron 1	Light response	1036-3100,3205-4021,4418-4711,5272-5509	Oryza sativa
367	PHE0000031	28	sorghum phyC with corn phyC intron 1	Light response	303-3710	Sorghum bicolor
368	PHE0000032	29	rice PF1	Light response	35-676	Oryza sativa
369	PHE0000033	30	rice GT2	Light response	58-2271	Oryza sativa
370	PHE0000034	31	Synechocystis biliverdin reductase	Light response	9-992	Synechocystis sp. PCC 6803
371	PHE0000038	32	corn cycD2.1	Cell division	125-1156	Zea mays
372	PHE0000039	33	corn nph1	Light response	415-3150	Zea mays
373	PHE0000040	34	corn hemoglobin 1	Stress tolerance	172-669	Zea mays
374	PHE0000043	35	rice cyclin 2	Cell division	148-1407	Oryza sativa
375	PHE0000044	36	rice cycC	Cell division	97-870	Oryza sativa
376	PHE0000045	37	rice cycB2	Cell division	74-1336	Oryza sativa
377	PHE0000046	38	rice cycA1	Cell division	97-1623	Oryza sativa
378	PHE0000047	39	rice cycB5	Cell division	292-361,1019-1347,1447-1572,1657-1908,2059-2217,2315-2493,3276-3432	Oryza sativa
379	PHE0000244	40	corn SVP-like	Flower development	177-860	Zea mays
380	PHE0000245	41	corn SVP-like	Flower development	93-791	Zea mays
381	PHE0000246	42	soy SVP-like	Flower development	96-713	Glycine max

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
382	PHE0000247	43	soy jointless-like	Flower development	60-674	Glycine max
383	PHE0000106	44	corn cycA1	Cell division	107-1633	Zea mays
384	PHE0000050	45	corn cycA2	Cell division	107-1222	Zea mays
385	PHE0000051	46	corn cycB2	Cell division	137-1408	Zea mays
386	PHE0000052	47	corn cycB5	Cell division	82-1518	Zea mays
387	PHE0000382	48	LIB3279-180-C9_FLI - maize cyclin III	Cell division	114-1385	Zea mays
388	PHE0000053	49	corn cycB4	Cell division	254-1579	Zea mays
389	PHE0000054	50	corn cycD3.2	Cell division	220-1380	Zea mays
390	PHE0000055	51	corn cycDx.1	Cell division	218-1180	Zea mays
391	PHE0000056	52	corn cycD1.1	Cell division	288-1334	Zea mays
392	PHE0000057	53	corn mt NDK - LIB189022Q1E1E9	Light response (Phytochrome?)	60-725	Zea mays
393	PHE0000058	54	corn cp NDK - 700479629	Light response	103-816	Zea mays
394	PHE0000059	55	corn NDK - LIB3597020Q1K6C3	Light response	49-495	Zea mays
395	PHE0000060	56	corn NDK - 700241377	Light response	162-608	Zea mays
396	PHE0000062	57	sRAD54 - with NLS	Homologous recombination	437-3556	Synechocystis sp. PCC 6803
397	PHE0000063	58	T4 endonuclease VII (gp49) - with NLS	Homologous recombination	603-1148	coliphage T4
398	PHE0000064	59	corn NDPK - fC-zmemLIB3957015Q1K6H6	Light response	91-624	Zea mays
399	PHE0000065	60	TOR1	Nitrogen assimilation	302-7714	Saccharomyces cerevisiae
400	PHE0000292	61	corn eIF-5A	Plant growth and development	85-564	Zea mays
401	PHE0000067	62	yeast eIF-5A	Plant growth and development	569-1042	Saccharomyces cerevisiae
402	PHE0000068	63	yeast deoxyhypusine synthase	Plant growth and development	173-1336	Saccharomyces cerevisiae
403	PHE0000069	64	yeast L5	Plant growth and development	987-1880	Saccharomyces cerevisiae
404	PHE0000070	65	yeast ornithine decarboxylase	Plant growth and development	576-1976	Saccharomyces cerevisiae
405	PHE0000071	66	rice exportin 4-like	Plant growth and development	501-750,1257-1417,1735-1800,3104-3218,3318-3427,3525-3620,7587-7744,7828-7915,8565-8669,8774-8878,9421-9450,9544-9656,9732-9819,9961-10180,11034-11164,12058-12204,12770-12898,12975-13073,13221-13259,14674-14823	Oryza sativa

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
406	PHE0000072	67	yeast S-adenosylmethionine decarboxylase	Plant growth and development	415-1605	Saccharomyces cerevisiae
407	PHE0000073	68	corn S-adenosylmethionine decarboxylase 1	Plant growth and development	268-1365	Zea mays
408	PHE0000074	69	corn S-adenosylmethionine decarboxylase 2	Plant growth and development	581-1780	Zea mays
409	PHE0000075	70	retinoblastoma-related protein 1	Cell division	37-2634	Zea mays
410	PHE0000076	71	C1 protein	Cell division	49-843	Wheat dwarf virus
411	PHE0000077	72	yeast flavohemoglobin - mitochondrial	Nitric oxide signaling	1695-2894	Saccharomyces cerevisiae
412	PHE0000009	73	Arabidopsis G975	Water use efficiency	58-654	Arabidopsis thaliana
413	PHE0000079	74	CUT1	Water use efficiency	372-1082,1176-1946	Oryza sativa
414	PHE0000082	75	corn cycB3	Cell division	88-1425	Zea mays
415	PHE0000083	76	PDR5	Disease resistance (cercosporin tolerance)	1552-6087	Saccharomyces cerevisiae
416	PHE0000084	77	rice cyclin H	Cell division	235-1227	Oryza sativa
417	PHE0000085	78	rice cdc2+/CDC28-related protein kinase	Cell division	173-1447	Oryza sativa
418	PHE0000086	79	Cdk-activating kinase 1	Cell division	14-1240	Glycine max
419	PHE0000089	80	CHL1	Nitrogen uptake/Seed development	85-1857	Arabidopsis thaliana
420	PHE0000090	81	NTR1	Nitrogen uptake/Seed development	144-1898	Oryza sativa
421	PHE0000091	82	Zm SET domain 2	Seed development	101-1009	Zea mays
422	PHE0000092	83	Zm SET domain 1	Seed development	528-1544	Zea mays
423	PHE0000095	84	HSF1	Heat tolerance/Water use efficiency	1017-3518	Saccharomyces cerevisiae
424	PHE0000096	85	Zm HSP101	Heat tolerance/Water use efficiency	436-1773,1878-2159,2281-2621,2711-2990,3079-3276,3371-3670	Zea mays
425	PHE0000098	86	E. coli clpB	Heat tolerance/Water use efficiency	557-3130	Escherichia coli
426	PHE0000099	87	Synechocystis clpB	Heat tolerance/Water use efficiency	316-2931	Synechocystis sp. PCC 6803

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
427	PHE0000100	88	Xylella clpB	Heat tolerance/Water use efficiency	187-2769	Xylella fastidiosa
428	PHE0000101	89	corn cycD3.1	Cell division	250-1422	Zea mays
429	PHE0000102	90	AnFPPS (farnesyl-pyrophosphate synthetase)	Glyphosphate tolerance	146-1186	Emericella nidulans
430	PHE0000103	91	OsFPPS	Glyphosphate tolerance	42-1103	Oryza sativa
431	PHE0000104	92	700331819_FLI - corn FPPS 2	Glyphosphate tolerance	313-1377	Zea mays
432	PHE0000105	93	corn cycD1.2	Cell division	229-1275	Zea mays
433	PHE0000107	94	corn cycD1.3	Cell division	206-1252	Zea mays
434	PHE0000108	95	ASH1	Stress tolerance	61-801	Arabidopsis thaliana
435	PHE0000109	96	rice ASH1-like1	Stress tolerance	136-1008	Oryza sativa
436	PHE0000110	97	rice MtN2-like	Stress tolerance	425-464,546-582,672-783,812-898,988-1149,1556-1675,1776-1952	Oryza sativa
437	PHE0000111	98	PAS domain kinase	Light response	358-2613	Zea mays
438	PHE0000114	99	Su(var) 3-9-like	Seed development	71-814	Zea mays
439	PHE0000115	100	Receiver domain (RR3-like) 7	Cell division	277-1002	Zea mays
440	PHE0000116	101	Receiver domain (ARR2-like) 1	Cell division	188-2245	Zea mays
441	PHE0000117	102	Receiver domain (TOC1-like) 2	Cell division	112-2238	Zea mays
442	PHE0000118	103	Receiver domain (TOC1-like) 3	Cell division	84-1976	Zea mays
443	PHE0000119	104	Receiver domain (ARR2-like) 4	Cell division	39-1931	Zea mays
444	PHE0000120	105	Receiver domain (RR11-like) 5	Cell division	61-1812	Zea mays
445	PHE0000121	106	Receiver domain (RR3-like) 6	Cell division	391-1116	Zea mays
446	PHE0000122	107	Receiver domain (RR3-like) 8	Cell division	335-1066	Zea mays
447	PHE0000123	108	Receiver domain 9	Cell division	55-759	Zea mays
448	PHE0000124	109	ZmRR2	Cell division	154-624	Zea mays
449	PHE0000125	110	Receiver domain (TOC1-like) 10	Cell division	374-722,791-2019	Zea mays
450	PHE0000126	111	corn HY5-like	Light response	32-541	Zea mays
451	PHE0000127	112	scarecrow 1 (PAT1-like)	Light response	295-1929	Zea mays
452	PHE0000128	113	scarecrow 2	Light response	153-1934	Zea mays
453	PHE0000133	114	G protein b subunit	Plant growth and development/Stress tolerance	90-1229	Zea mays
454	PHE0000152	115	14-3-3-like protein 2	Nitrogen assimilation	85-861	Glycine max

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
455	PHE0000153	116	14-3-3-like protein D	Nitrogen assimilation	42-824	Glycine max
456	PHE0000154	117	14-3-3 protein 1	Nitrogen assimilation	49-834	Glycine max
457	PHE0000155	118	Rice FAP1-like protein	Nitrogen assimilation	654-1862,2310-2426,3407-3492,3590-3752,3845-3890,4476-4522,4985-5191,5306-5392,5473-5640	Oryza sativa
458	PHE0000156	119	rice TAP42-like	Nitrogen assimilation	199-1338	Oryza sativa
459	PHE0000158	120	BMH1	Nitrogen assimilation	79-882	Saccharomyces cerevisiae
460	PHE0000159	121	rice chloroplastic fructose-1,6-bisphosphatase	Yield associated genes	41-1261	Oryza sativa
461	PHE0000160	122	E. coli fructose-1,6-bisphosphatase	Yield associated genes	208-1206	Escherichia coli
462	PHE0000161	123	Synechocystis fructose-1,6-bisphosphatase F-I	Yield associated genes	1-1164	Synechocystis sp. PCC 6803
463	PHE0000162	124	Synechocystis fructose-1,6-bisphosphatase F-II	Yield associated genes	480-1523	Synechocystis sp. PCC 6803
464	PHE0000164	125	Yeast RPT5	Yield associated genes	883-2187	Saccharomyces cerevisiae
465	PHE0000165	126	Yeast RRP5	Yield associated genes	331-5520	Saccharomyces cerevisiae
466	PHE0000166	127	Rice CBP-like gene	Yield associated genes	277-436,479-1524,1790-2065,2150-2425,3134-3262,3380-3580,3683-3825,3905-4190,4294-4433,4711-4789,4874-4929,5754-5946	Oryza sativa
467	PHE0000167	128	rice BAB09754	Yield associated genes	616-903,1848-1940,2046-2165,2254-2355,2443-2693,2849-2994,3165-3363,3475-4141,4438-4770,5028-5309	Oryza sativa
468	PHE0000168	129	LIB3061-001-H7_FLI	Yield associated genes	309-1037	Zea mays
469	PHE0000169	130	maize p23	Heat tolerance/Water use efficiency	106-708	Zea mays
470	PHE0000170	131	maize cyclophilin	Heat tolerance/Water use efficiency	99-1757	Zea mays
471	PHE0000172	132	yeast SIT1	Heat tolerance/Water use efficiency	361-2130	Saccharomyces cerevisiae

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
472	PHE0000173	133	yeast CNS1	Heat tolerance/Water use efficiency	762-1919	Saccharomyces cerevisiae
473	PHE0000176	134	RNAse S	Phosphate uptake	85-771	Zea mays
474	PHE0000177	135	maize ecto-apyrase	Phosphate uptake	210-2312	Zea mays
475	PHE0000178	136	PHO5	Phosphate uptake	1-1404	Saccharomyces cerevisiae
476	PHE0000179	137	high affinity phosphate translocator	Phosphate uptake	105-1703	Glycine max
477	PHE0000180	138	high affinity phosphate translocator	Phosphate uptake	128-1750	Zea mays
478	PHE0000181	139	Xylella citrate synthase	Phosphate uptake	256-1545	Xylella fastidiosa
479	PHE0000182	140	E. coli citrate synthase	Phosphate uptake	309-1592	Escherichia coli
480	PHE0000183	141	rice citrate synthase	Phosphate uptake	105-1523	Oryza sativa
481	PHE0000184	142	citrate synthase	Phosphate uptake	56-1564	Zea mays
482	PHE0000185	143	citrate synthase	Phosphate uptake	153-1691	Glycine max
483	PHE0000186	144	maize ferritin 2	Stress tolerance	3-758	Zea mays
484	PHE0000187	145	maize ferritin 1	Stress tolerance	34-795	Zea mays
485	PHE0000188	146	E. coli cytoplasmic ferritin	Stress tolerance	245-742	Escherichia coli
486	PHE0000190	147	corn LEA3	Cold tolerance	171-755	Zea mays
487	PHE0000192	148	soy HSF	Heat tolerance/Water use efficiency	23-1114	Glycine max
488	PHE0000193	149	soy HSF	Heat tolerance/Water use efficiency	93-992	Glycine max
489	PHE0000204	150	deoxyhypusine synthase	Plant growth and development	26-1129	Glycine max
490	PHE0000219	151	thylakoid carbonic anhydrase, cah3	Photosynthesis	62-994	Chlamydomonas reinhardtii
491	PHE0000216	152	thylakoid carbonic anhydrase, ecaA	Photosynthesis	49-843	Nostoc PCC7120
492	PHE0000217	153	Chlamydomonas reinhardtii envelope protein LIP-36G1	Photosynthesis	156-1232	Chlamydomonas reinhardtii
493	PHE0000218	154	psbO transit peptide::Synechococcus sp. PCC 7942 ictB	Photosynthesis	271-1674	Synechococcus sp. PCC 7942
494	PHE0000220	155	corn RNase PH	Disease resistance	86-805	Zea mays
495	PHE0000221	156	SKI2	Disease resistance	1351-5211	Saccharomyces cerevisiae
496	PHE0000222	157	SKI3	Disease resistance	793-5091	Saccharomyces cerevisiae
497	PHE0000223	158	SKI4	Disease resistance	323-1201	Saccharomyces cerevisiae
498	PHE0000224	159	SKI6	Disease resistance	1007-1747	Saccharomyces cerevisiae
499	PHE0000225	160	SKI7	Disease resistance	279-2519	Saccharomyces cerevisiae

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
500	PHE0000226	161	rice SKI7-like	Disease resistance	464-884,1132-1287,2103-2252,2353-2487,2957-3288,3399-3509,3596-4095,4350-4518,4783-5022,5097-5228,5315-5449	Oryza sativa
501	PHE0000228	162	Synechocystis cobA w cp transit peptide	Nitrogen metabolism	70-801	Synechocystis sp. PCC 6803
502	PHE0000229	163	Xylella tetrapyrrole methylase with transit peptide	Nitrogen metabolism	1-774	Xylella fastidiosa
503	PHE0000230	164	maize uroporphyrinogen III methyltransferase	Nitrogen metabolism	15-1286	Zea mays
504	PHE0000231	165	nucellin-like protein	Seed development	122-1594	Zea mays
505	PHE0000232	166	nucellin-like protein	Seed development	76-1605	Zea mays
506	PHE0000233	167	nucellin-like protein	Seed development	195-1628	Zea mays
507	PHE0000234	168	soy LEA protein	Cold tolerance	6-704	Glycine max
508	PHE0000235	169	dehydrin-like protein	Cold tolerance	33-710	Glycine max
509	PHE0000237	170	dehydrin 3	Cold tolerance	84-584	Zea mays
510	PHE0000238	171	probable lipase	Cold tolerance	98-967	Zea mays
511	PHE0000239	172	yeast GRE1	Cold tolerance	1024-1527	Saccharomyces cerevisiae
512	PHE0000240	173	yeast STF2	Cold tolerance	683-934	Saccharomyces cerevisiae
513	PHE0000241	174	yeast SIP18	Cold tolerance	376-855	Saccharomyces cerevisiae
514	PHE0000242	175	yeast YBM6	Cold tolerance	744-1130	Saccharomyces cerevisiae
515	PHE0000243	176	yeast HSP12	Cold tolerance	282-611	Saccharomyces cerevisiae
516	PHE0000249	177	corn allene oxide synthase	Stress tolerance/Disease resistance	111-1556	Zea mays
517	PHE0000250	178	corn COI1-like	Stress tolerance/Disease resistance	139-1911	Zea mays
518	PHE0000251	179	corn TIR1-like	Plant growth and development	113-1906	Zea mays
519	PHE0000252	180	corn COI1-like	Stress tolerance/Disease resistance	130-1923	Zea mays
520	PHE0000253	181	COI1-like	Stress tolerance/Disease resistance	389-2368	Zea mays
521	PHE0000254	182	F-box protein	Plant growth and development	123-1304	Glycine max
522	PHE0000255	183	F-box protein	Plant growth and development	228-1916	Glycine max

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
523	PHE0000256	184	corn 1-aminocyclopropane-1-carboxylate oxidase	Stress tolerance/Disease resistance	61-1011	Zea mays
524	PHE0000257	185	rice 1-aminocyclopropane-1 carboxylate synthase	Stress tolerance/Disease resistance	2-1465	Oryza sativa
525	PHE0000260	186	S52650 - Synechocystis desB	Cold tolerance	643-1719	Synechocystis sp. PCC 6803
526	PHE0000261	187	yeast glutamate decarboxylase	Stress tolerance	33-1790	Saccharomyces cerevisiae
527	PHE0000262	188	cytochrome P450-like protein	Plant growth and development	29-1495	Zea mays
528	PHE0000263	189	cytochrome P450	Plant growth and development	141-1637	Zea mays
529	PHE0000264	190	cytochrome P450-like	Plant growth and development	104-1657	Zea mays
530	PHE0000265	191	CYP90 protein	Plant growth and development	81-1589	Zea mays
531	PHE0000266	192	cytochrome P450 DWARF3	Plant growth and development	92-1648	Zea mays
532	PHE0000267	193	cytochrome P450	Plant growth and development	134-1543	Zea mays
533	PHE0000268	194	rice receptor protein kinase	Plant growth and development	183-476,706-735,2796-6734	Oryza sativa
534	PHE0000269	195	soy E2F-like	Cell division	80-1117	Glycine max
535	PHE0000270	196	nuclear matrix constituent protein	Cell division	243-3371	Zea mays
536	PHE0000271	197	OsE2F1	Cell division	93-1403	Oryza sativa
537	PHE0000272	198	corn GCR1	Cell division	74-1036	Zea mays
538	PHE0000273	199	soy mlo-like	Plant growth and development/Stress tolerance	15-1532	Glycine max
539	PHE0000274	200	soy mlo-like	Plant growth and development/Stress tolerance	48-1841	Glycine max
540	PHE0000275	201	rice G alpha 1	Plant growth and development/Stress tolerance	106-1248	Oryza sativa
541	PHE0000276	202	soy G-gamma subunit	Plant growth and development/Stress tolerance	210-536	Glycine max
542	PHE0000277	203	wheat G28-like	Disease resistance	65-877	Triticum aestivum
543	PHE0000279	204	sorghum proline permease	Nitrogen transport	16-1341	Sorghum bicolor
544	PHE0000280	205	rice AA transporter	Nitrogen transport	61-1485	Oryza sativa
545	PHE0000282	206	SET-domain protein-like	Seed development	478-3045	Zea mays
546	PHE0000283	207	scarecrow 6	Light response	520-2145	Zea mays
547	PHE0000284	208	menage a trois-like	Cell division	164-745	Zea mays
548	PHE0000286	209	oryzacystatin	Pest tolerance	108-527	Oryza sativa

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
549	PHE0000287	210	Similar to cysteine proteinase inhibitor	Pest tolerance	18-767	Oryza sativa
550	PHE0000288	211	cysteine proteinase inhibitor	Pest tolerance	135-461	Sorghum bicolor
551	PHE0000289	212	Zm-GRF1 (GA responsive factor)	Plant growth and development	96-1202	Zea mays
552	PHE0000290	213	ZmSE001-like	Flower development	253-2115	Zea mays
553	PHE0000291	214	deoxyhypusine synthase	Plant growth and development	54-1163	Zea mays
554	PHE0000293	215	gibberellin response modulator	Light response	131-2020	Zea mays
555	PHE0000294	216	scarecrow-like protein	Light response	266-1948	Zea mays
556	PHE0000295	217	ubiquitin-conjugating enzyme-like protein	Yield associated genes	114-599	Zea mays
557	PHE0000296	218	unknown protein recognized by PF01169	Yield associated genes	90-785	Zea mays
558	PHE0000297	219	26S protease regulatory subunit 6A homolog	Yield associated genes	57-1343	Oryza sativa
559	PHE0000298	220	rice p23 co-chaperone	Heat tolerance/Water use efficiency	68-706	Oryza sativa
560	PHE0000299	221	corn p23 co-chaperone	Heat tolerance/Water use efficiency	71-565	Zea mays
561	PHE0000300	222	rice p23 co-chaperone	Heat tolerance/Water use efficiency	124-642	Oryza sativa
562	PHE0000301	223	corn p23 co-chaperone	Heat tolerance/Water use efficiency	90-617	Zea mays
563	PHE0000302	224	putative purple acid phosphatase precursor	Phosphate uptake	22-1038	Oryza sativa
564	PHE0000303	225	acid phosphatase type 5	Phosphate uptake	143-1186	Zea mays
565	PHE0000304	226	aleurone ribonuclease	Phosphate uptake	47-814	Oryza sativa
566	PHE0000305	227	putative ribonuclease	Phosphate uptake	55-888	Zea mays
567	PHE0000306	228	S-like RNase	Phosphate uptake	15-770	Zea mays
568	PHE0000307	229	ribonuclease	Phosphate uptake	95-781	Zea mays
569	PHE0000308	230	helix-loop-helix protein (PIF3-like)	Light response	202-756	Zea mays
570	PHE0000309	231	SKI4-like protein	Disease resistance	36-632	Zea mays
571	PHE0000310	232	putative 3 exoribonuclease	Disease resistance	238-1098	Zea mays
572	PHE0000311	233	GF14-c protein	Nitrogen assimilation	81-848	Oryza sativa
573	PHE0000312	234	14-3-3-like protein	Nitrogen assimilation	6-785	Oryza sativa

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
574	PHE0000313	235	rice eIF-(iso)4F	Nitrogen assimilation	96-713	Oryza sativa
575	PHE0000314	236	rice eIF-4F	Nitrogen assimilation	46-726	Oryza sativa
576	PHE0000315	237	sorghum eIF-(iso)4F	Nitrogen assimilation	78-707	Sorghum bicolor
577	PHE0000316	238	sorghum eIF-4F	Nitrogen assimilation	9-668	Sorghum bicolor
578	PHE0000317	239	rice FIP37-like	Nitrogen assimilation	73-1128	Oryza sativa
579	PHE0000318	240	scarecrow 17	Light response	441-2102	Zea mays
580	PHE0000322	241	maize catalase-1	Stress tolerance	208-1683	Zea mays
581	PHE0000323	242	maize catalase-3	Stress tolerance	30-1511	Zea mays
582	PHE0000324	243	ascorbate peroxidase	Stress tolerance	197-1063	Zea mays
583	PHE0000325	244	corn GDI	Stress tolerance	57-1397	Zea mays
584	PHE0000326	245	soy GDI	Stress tolerance	45-1418	Glycine max
585	PHE0000327	246	corn rho GDI	Stress tolerance	463-1203	Zea mays
586	PHE0000328	247	basic blue copper protein	Stress tolerance	13-408	Zea mays
587	PHE0000329	248	plantacyanin	Stress tolerance	109-489	Zea mays
588	PHE0000330	249	basic blue copper protein	Stress tolerance	83-463	Glycine max
589	PHE0000331	250	Similar to blue copper protein precursor	Stress tolerance	323-868	Zea mays
590	PHE0000332	251	lamin	Stress tolerance	62-646	Zea mays
591	PHE0000333	252	fC-zmfl700551169a-allyl alcohol dehydrogenase	Stress tolerance	56-1105	Zea mays
592	PHE0000334	253	allyl alcohol dehydrogenase	Stress tolerance	103-1128	Glycine max
593	PHE0000335	254	allyl alcohol dehydrogenase	Stress tolerance	6-1079	Zea mays
594	PHE0000336	255	quinone oxidoreductase	Stress tolerance	47-1051	Zea mays
595	PHE0000337	256	E. nidulans cysA - AF029885	Stress tolerance	384-1961	Emericella nidulans
596	PHE0000338	257	BAA18167 - Synechocystis cysE	Stress tolerance	801-1547	Synechocystis sp. PCC 6803
597	PHE0000339	258	Synechocystis thiol-specific antioxidant protein - BAA10136	Stress tolerance	36-638	Synechocystis sp. PCC 6803
598	PHE0000340	259	yeast TSA2 - NP_010741	Stress tolerance	108-698	Saccharomyces cerevisiae
599	PHE0000341	260	yeast mTPx - Z35825	Stress tolerance	730-1512	Saccharomyces cerevisiae
600	PHE0000343	261	yeast TPx III - NP_013210	Stress tolerance	657-1187	Saccharomyces cerevisiae
601	PHE0000345	262	soy putative 2-cys peroxiredoxin	Stress tolerance	160-939	Glycine max
602	PHE0000346	263	soy peroxiredoxin	Stress tolerance	104-745	Glycine max

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603	PHE0000347	264	heat shock protein 26, plastid-localized	Stress tolerance	117-836	Zea mays
604	PHE0000349	265	heat shock protein	Stress tolerance	112-735	Zea mays
605	PHE0000350	266	low molecular weight heat shock protein	Stress tolerance	28-690	Zea mays
606	PHE0000351	267	18kDa heat shock protein	Stress tolerance	103-597	Zea mays
607	PHE0000352	268	heat shock protein 16.9	Stress tolerance	229-690	Zea mays
608	PHE0000353	269	HSP21-like protein	Stress tolerance	73-696	Zea mays
609	PHE0000354	270	Opt1p - NP_012323	Stress tolerance	508-2904	Saccharomyces cerevisiae
610	PHE0000355	271	SVCT2-like permease	Stress tolerance	220-1779	Zea mays
611	PHE0000356	272	SVCT2-like permease	Stress tolerance	34-1632	Zea mays
612	PHE0000357	273	maize tubby-like	Plant growth and development	519-1958	Zea mays
613	PHE0000358	274	maize tubby-like	Plant growth and development	517-1269	Zea mays
614	PHE0000359	275	soy HMG CoA synthase	Stress tolerance	80-1441	Glycine max
615	PHE0000360	276	yeast HMGS - X96617	Stress tolerance	220-1695	Saccharomyces cerevisiae
616	PHE0000361	277	PAT1-like scarecrow 9	Light response	191-1900	Zea mays
617	PHE0000362	278	CDC28-related protein kinase	Cell division	198-1484	Zea mays
618	PHE0000385	279	H+ transporting ATPase	Metabolite transport	176-2836	Zea mays
619	PHE0000386	280	cation-transporting ATPase	Metabolite transport	222-2168	Zea mays
620	PHE0000387	281	yeast DRS2 (ALA1-like) - L01795	Metabolite transport	170-4237	Saccharomyces cerevisiae
621	PHE0000388	282	S. pombe ALA1-like-CAA21897	Metabolite transport	56-3832	Schizosaccharomyces pombe
622	PHE0000389	283	rice ALA1-like 1 - BAA89544	Metabolite transport	47-1538,1619-1925,3116-3824,3920-4043,4143-4362,4590-5048,5937-6153	Oryza sativa
623	PHE0000390	284	rice chloroplastic sedoheptulose-1,7-bisphosphatase-	Photosynthesis/Carbon partitioning	136-1311	Oryza sativa
624	PHE0000391	285	rice cytosolic fructose-1,6-bisphosphatase	Photosynthesis/Carbon partitioning	171-1187	Oryza sativa
625	PHE0000392	286	Wheat sedoheptulose-1,7-bisphosphatase	Photosynthesis/Carbon partitioning	14-1192	Triticum aestivum
626	PHE0000394	287	sedoheptulose-1,7-bisphosphatase	Photosynthesis/Carbon partitioning	90-1238	Chlorella sorokiniana
627	PHE0000395	288	soy phantastica	Plant growth and development	275-1345	Glycine max
628	PHE0000396	289	soy phantastica 2	Plant growth and development	178-1260	Glycine max

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
629	PHE0000397	290	maize rough sheath 1	Plant growth and development	92-1144	Zea mays
630	PHE0000398	291	soy lg3-like 1	Plant growth and development	103-1026	Glycine max
631	PHE0000399	292	soy rough sheath1-like 1	Plant growth and development	144-1076	Glycine max
632	PHE0000400	293	soy G559-like	Plant growth and development	301-1560	Glycine max
633	PHE0000401	294	soy G1635-like 1	Plant growth and development	28-888	Glycine max
634	PHE0000402	295	rice amino acid transporter-like protein	Nitrogen transport	89-1426	Oryza sativa
635	PHE0000403	296	corn amino acid permease	Nitrogen transport	116-1453	Zea mays
636	PHE0000404	297	rice proline transport protein	Nitrogen transport	313-1731	Oryza sativa
637	PHE0000412	298	corn monosaccharide transporter 1	Sucrose transport	75-1643	Zea mays
638	PHE0000413	299	soy monosaccharide transporter 3	Sucrose transport	132-1685	Glycine max
639	PHE0000414	300	corn monosaccharide transporter 3	Sucrose transport	141-1670	Zea mays
640	PHE0000415	301	soy monosaccharide transporter 1	Sucrose transport	160-1899	Glycine max
641	PHE0000416	302	corn monosaccharide transporter 6	Sucrose transport	74-1690	Zea mays
642	PHE0000418	303	corn monosaccharide transporter 4	Sucrose transport	146-1744	Zea mays
643	PHE0000419	304	soy monosaccharide transporter 2	Sucrose transport	63-1505	Glycine max
644	PHE0000420	305	soy sucrose transporter	Sucrose transport	63-1595	Glycine max
645	PHE0000421	306	corn sucrose transporter 2	Sucrose transport	76-1599	Zea mays
646	PHE0000422	307	corn monosaccharide transporter 8	Sucrose transport	201-1763	Zea mays
647	PHE0000423	308	corn monosaccharide transporter 7	Sucrose transport	93-1634	Zea mays
648	PHE0000425	309	soy isoflavone synthase	Stress tolerance	45-1607	Glycine max
649	PHE0000426	310	soy ttg1-like 2	Stress tolerance	52-1059	Glycine max
650	PHE0000427	311	GATE5 - corn SPA1-like 1	Light response	227-3139	Zea mays
651	PHE0000428	312	corn PIF3-like	Light response	173-856	Zea mays
652	PHE0000429	313	soy Athb-2-like 1	Light response	78-932	Glycine max
653	PHE0000430	314	corn SUB1-like 1	Light response	44-1954	Zea mays
654	PHE0000431	315	soy GH3 protein	Light response	42-1820	Glycine max
655	PHE0000432	316	corn 12-oxophytodienoate reductase 1	Stress tolerance/Disease resistance	128-1240	Zea mays

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656	PHE0000433	317	corn 12-oxo-phytodienoate reductase-like 3	Stress tolerance/Disease resistance	166-1242	Zea mays
657	PHE0000434	318	corn 12-oxophytodienoate reductase-like 4	Stress tolerance/Disease resistance	92-1210	Zea mays
658	PHE0000435	319	corn hydroperoxide lyase	Stress tolerance/Disease resistance	83-1594	Zea mays
659	PHE0000436	320	rice cns1-like	Heat tolerance/Water use efficiency	121-1242	Oryza sativa
660	PHE0000437	321	corn HCH1-like 1	Heat tolerance/Water use efficiency	42-1100	Zea mays
661	PHE0000438	322	corn HOP-like 1	Heat tolerance/Water use efficiency	88-1830	Zea mays
662	PHE0000439	323	corn HOP-like 2	Heat tolerance/Water use efficiency	65-1261	Zea mays
663	PHE0000440	324	rice CHIP-like 1	Heat tolerance/Water use efficiency	121-939	Oryza sativa
664	PHE0000441	325	corn CHIP-like 2	Heat tolerance/Water use efficiency	115-939	Zea mays
665	PHE0000451	326	wheat SVP-like 1	Flower development	149-736	Triticum aestivum
666	PHE0000452	327	corn SVP-like 3	Flower development	75-749	Zea mays
667	PHE0000453	328	corn SVP-like 5	Flower development	304-774,956-1219	Zea mays
668	PHE0000454	329	fC-zmhuLIB3062-044-Q1-K1-B8	Yield associated genes	113-853	Zea mays
669	PHE0000455	330	corn E4/E8 binding protein-like	Yield associated genes	253-2259	Zea mays
670	PHE0000469	331	yeast YKL091c - Z28091	Stress tolerance	110-1042	Saccharomyces cerevisiae
671	PHE0000470	332	corn Ssh1-like protein 1	Stress tolerance	57-1037	Zea mays
672	PHE0000471	333	corn Ssh1-like protein 3	Stress tolerance	89-841	Zea mays
673	PHE0000472	334	corn Ssh1-like protein 4	Stress tolerance	309-1196	Zea mays
674	PHE0000473	335	soy Ssh1-like protein 2 [ssh2]	Stress tolerance	209-976	Glycine max
675	PHE0000484	336	soy JMT-like protien 1	Stress tolerance/Disease resistance	26-1135	Glycine max

PEP SEQ ID NO	Phe ID	NUC SEQ ID NO	Gene Name	Gene Effect	CODING SEQUENCE	Species
676	PHE0000485	337	corn JMT-like protein 1	Stress tolerance/Disease resistance	39-1184	Zea mays
677	PHE0000486	338	corn JMT-like protein 2	Stress tolerance/Disease resistance	63-1208	Zea mays
678	PHE0000017	339	corn AAA-ATPase 1	Plastid division	184-2214	Zea mays

Transgenic plants having enhanced phenotypes are identified from populations of plants transformed as described herein by evaluating the phenotype in a variety of assays to detect an enhanced phenotype. These assays also may take many forms, including but not limited to, analyses to detect changes in the chemical composition, morphology, biomass or physiological responses of the plant to stress conditions. Enhanced physiological properties in transgenic plants of the present invention may be identified by evaluation of responses to stress conditions, for example in assays using imposed stress conditions to detect improved responses to water stress, nitrogen deficiency, cold growing conditions, pathogen or insect attack or light deficiency, or alternatively, under naturally present stress conditions, for example under field conditions. Enhanced chemical compositions, such as nutritional composition of grain, may be detected by analysis, for example, of composition and content of seed protein, free amino acids, oil, free fatty acids, starch or tocopherols. Biomass measures may be made on greenhouse or field grown plants and may include such measurements as plant height, stem diameter, root and shoot dry weights, and, for corn plants, ear length and diameter

Phenotypic data on morphological changes may be collected by visual observation during the process of plant regeneration as well as in regenerated plants transferred to soil. Such phenotypic data includes characteristics such as normal plants, bushy plants, taller plants, thicker stalks, narrow leaves, striped leaves, knotted phenotype, chlorosis, albino, anthocyanin production, or altered tassels, ears or roots. Other enhanced phenotypes may be identified by measurements taken under field conditions, such as days to pollen shed, days to silking, leaf extension rate, chlorophyll content, leaf temperature, stand, seedling vigor, internode length, plant height, leaf number, leaf area, tillering, brace roots, stay green, stalk lodging, root lodging, plant health, barreness/prolificacy, green snap, and pest resistance. In addition, phenotypic

characteristics of harvested grain may be evaluated, including number of kernels per row on the ear, number of rows of kernels on the ear, kernel abortion, kernel weight, kernel size, kernel density and physical grain quality.

To confirm hybrid yield in transgenic corn plants expressing genes of the present invention, it may be desirable to test hybrids over multiple years at multiple locations in a geographical location where maize is conventionally grown, e.g. in Iowa, Illinois or other locations in the midwestern United States, under "normal" field conditions as well as under stress conditions, e.g. under drought or population density stress.

Of particular interest in the present invention are corn and soybean plants. Other plants of interest in the present invention for production of transgenic seed that can be grown to provide plants having enhanced properties include, without limitation, cotton, canola, wheat, sunflower, sorghum, alfalfa, barley, millet, rice, tobacco, fruit and vegetable crops, and turfgrass.

Protein and Polypeptide Molecules

Polypeptides considered in the present invention are entire proteins or at least a sufficient portion of the entire protein to impart the relevant biological activity of the protein, e.g. enhanced plant phenotype. The term "protein" also includes molecules consisting of one or more polypeptide chains. Thus, a polypeptide useful in the present invention may constitute an entire protein having the desired biological activity, or may constitute a portion of an oligomeric protein having multiple polypeptide chains. Polypeptides useful for generation of transgenic plants having enhanced properties include the polypeptides provided herein as SEQ ID NO:340 through SEQ ID NO:678, as well as homologs of such polypeptides.

Homologs of the polypeptides of the present invention may be identified by comparison of the amino acid sequence of the polypeptide to amino acid sequences of polypeptides from the same or different plant sources, e.g. manually or by using known homology-based search algorithms such as those commonly known and referred to as BLAST, FASTA, and Smith-Waterman. As used herein, a homolog is a peptide from the same or a different organism that performs the same biological function as the polypeptide to which it is compared. An orthologous relation between two organisms is not necessarily manifest as a one-to-one correspondence between two genes, because a gene can be duplicated or deleted after organism phylogenetic separation, such as speciation. For a given polypeptide, there may be no ortholog

or more than one ortholog. Other complicating factors include alternatively spliced transcripts from the same gene, limited gene identification, redundant copies of the same gene with different sequence lengths or corrected sequence. A local sequence alignment program, e.g. BLAST, can be used to search a database of sequences to find similar sequences, and the summary Expectation value (E-value) used to measure the sequence base similarity. As a polypeptide hit with the best E-value for a particular organism may not necessarily be an ortholog or the only ortholog, a reciprocal BLAST search is used in the present invention to filter hit sequences with significant E-values for ortholog identification. The reciprocal BLAST entails search of the significant hits against a database of polypeptide sequences from the base organism that are similar to the sequence of the query polypeptide. A hit is a likely ortholog, when the reciprocal BLAST's best hit is the query polypeptide itself or a polypeptide encoded by a duplicated gene after speciation. Thus, homolog is used herein to described polypeptides that are assumed to have functional similarity by inference from sequence base similarity. Homologs of the polypeptides of the present invention are described in Table 2 provided on the CD-ROM provided herewith, and disclosed as SEQ ID NO:679 through SEQ ID NO:24149.

A further aspect of the invention comprises functional homolog proteins which differ in one or more amino acids from those of a polypeptide provided herein as the result of one or more of the well-known conservative amino acid substitutions, e.g. valine is a conservative substitute for alanine and threonine is a conservative substitute for serine. Conservative substitutions for an amino acid within the native polypeptide sequence can be selected from other members of a class to which the naturally occurring amino acid belongs. Representative amino acids within these various classes include, but are not limited to: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; and (4) neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. Conserved substitutes for an amino acid within a native amino acid sequence can be selected from other members of the group to which the naturally occurring amino acid belongs. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and

glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Naturally conservative amino acids substitution groups are: valine-leucine, valine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, aspartic acid-glutamic acid, and asparagine-glutamine. A further aspect of the invention comprises polypeptides that differ in one or more amino acids from those of a described protein sequence as the result of deletion or insertion of one or more amino acids in a native sequence.

Homologs of the polypeptides provided herein will generally demonstrate significant identity with the polypeptides provided herein. Of particular interest are polypeptides having at least 50% sequence identity, more preferably at least about 70% sequence identity or higher, e.g. at least about 80% sequence identity with an amino acid sequence of SEQ ID NO:1 through SEQ ID NO:339. Of course useful polypeptides also include those with higher identity to such a polypeptide sequence, e.g. 90% to 99% identity. Identity of protein homologs is determined by optimally aligning the amino acid sequence of a putative protein homolog with a defined amino acid sequence and by calculating the percentage of identical and conservatively substituted amino acids over the window of comparison. Preferentially, the window of comparison for determining identity is the entire polypeptide sequence disclosed herein, e.g. the full sequence of any of SEQ ID NO:340 through SEQ ID NO:678.

Recombinant Polynucleotides

The present invention contemplates the use of polynucleotides effective for imparting an enhanced phenotype to transgenic plants expressing said polynucleotides. Exemplary polynucleotides for use in the present invention are listed in Table 4 above and provided herein as SEQ ID NO:1 through SEQ ID NO:339. A subset of the nucleic molecules of this invention includes fragments of the disclosed polynucleotides consisting of oligonucleotides of at least 15, preferably at least 16 or 17, more preferably at least 18 or 19, and even more preferably at least 20 or more, consecutive nucleotides. Such oligonucleotides are fragments of the larger molecules having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO:339, and find use, for example as probes and primers for detection of the polynucleotides of the present invention.

Also of interest in the present invention are variants of the polynucleotides provided herein. Such variants may be naturally occurring, including homologous polynucleotides from the same or a different species, or may be non-natural variants, for example polynucleotides synthesized using chemical synthesis methods, or generated using recombinant DNA techniques. Degeneracy of the genetic code provides the possibility to substitute at least one base of the protein encoding sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, a polynucleotide useful in the present invention may have any base sequence that has been changed from SEQ ID NO:1 to SEQ ID NO:339 by substitution in accordance with degeneracy of the genetic code.

Homologs of the polynucleotides provided herein will generally demonstrate significant identity with the polynucleotides provided herein. A polynucleotide of the present invention is substantially identical to a reference polynucleotide if, when the sequences of the polynucleotides are optimally aligned there is about 60% nucleotide equivalence; more preferably 70%; more preferably 80% equivalence; more preferably 85% equivalence; more preferably 90%; more preferably 95%; and/or more preferably 98% or 99% equivalence over a comparison window. A comparison window is preferably at least 50-100 nucleotides, and more preferably is the entire length of the polynucleotide provided herein. Optimal alignment of sequences for aligning a comparison window may be conducted by algorithms; preferably by computerized implementations of these algorithms (for example, the Wisconsin Genetics Software Package Release 7.0-10.0, Genetics Computer Group, 575 Science Dr., Madison, WI). The reference polynucleotide may be a full-length molecule or a portion of a longer molecule. Preferentially, the window of comparison for determining polynucleotide identity of protein encoding sequences is the entire coding region.

In a preferred embodiment, a polynucleotide of the present invention is operatively linked in a recombinant polynucleotide to a promoter functional in a plant to provide for expression of the polynucleotide in the sense orientation such that a desired polypeptide is produced. Also considered are embodiments wherein a polynucleotide is operatively linked to a promoter functional in a plant to provide for expression of the polynucleotide in the antisense orientation such that a complementary copy of at least a portion of an mRNA native to the target plant host is produced. Such a transcript may contain both sense and antisense regions of a polynucleotide, for example where RNAi methods are used for gene suppression.

Recombinant polynucleotides of the present invention are assembled in recombinant DNA constructs using methods known to those of ordinary skill in the art. Thus, transgenic DNA constructs used for transforming plant cells will comprise a polynucleotide one desires to introduce into a target plant. Such constructs will also typically comprise a promoter operatively linked to said polynucleotide to provide for expression in the target plant. Other construct components may include additional regulatory elements, such as 5' or 3' untranslated regions (such as polyadenylation sites), intron regions, and transit or signal peptides.

Numerous promoters that are active in plant cells have been described in the literature. These include promoters present in plant genomes as well as promoters from other sources, including nopaline synthase (NOS) promoter and octopine synthase (OCS) promoters carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*, caulimovirus promoters such as the cauliflower mosaic virus or figwort mosaic virus promoters. For instance, see U.S. Patents No. 5,858,742 and 5,322,938 which disclose versions of the constitutive promoter derived from cauliflower mosaic virus (CaMV35S), US Patent No. 5,378,619 which discloses a Figwort Mosaic Virus (FMV) 35S promoter, U.S. Patent 6,437,217 which discloses a maize RS81 promoter, U.S. Patent 5,641,876 which discloses a rice actin promoter, U.S. Patent 6,426,446 which discloses a maize RS324 promoter, U.S. Patent 6,429,362 which discloses a maize PR-1 promoter, U.S. Patent 6,232,526 which discloses a maize A3 promoter, U.S. Patent 6,177,611 which discloses constitutive maize promoters, U.S. Patent 6,433,252 which discloses a maize L3 oleosin promoter, U.S. Patent 6,429,357 which discloses a rice actin 2 promoter and intron, U.S. Patent 5,837,848 which discloses a root specific promoter, U.S. Patent 6,084,089 which discloses cold inducible promoters, U.S. Patent 6,294,714 which discloses light inducible promoters, U.S. Patent 6,140,078 which discloses salt inducible promoters, U.S. Patent 6,252,138 which discloses pathogen inducible promoters, U.S. Patent 6,175,060 which discloses phosphorus deficiency inducible promoters, U.S. Patent Application Publication 2002/0192813A1 which discloses 5', 3' and intron elements useful in the design of effective plant expression vectors, U.S. patent application Serial No. 09/078,972 which discloses a coixin promoter, U.S. patent application Serial No. 09/757,089 which discloses a maize chloroplast aldolase promoter, all of which are incorporated herein by reference. These and numerous other promoters that function in plant cells are known to those skilled in the art and available for use in recombinant

polynucleotides of the present invention to provide for expression of desired genes in transgenic plant cells.

Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression. Such enhancers are known in the art. By including an enhancer sequence with such constructs, the expression of the selected protein may be enhanced. These enhancers often are found 5' to the start of transcription in a promoter that functions in eukaryotic cells, but can often be inserted in the forward or reverse orientation 5' or 3' to the coding sequence. In some instances, these 5' enhancing elements are introns. Deemed to be particularly useful as enhancers are the 5' introns of the rice actin 1 and rice actin 2 genes. Examples of other enhancers that can be used in accordance with the invention include elements from the CaMV 35S promoter, octopine synthase genes, the maize alcohol dehydrogenase gene, the maize shrunken 1 gene and promoters from non-plant eukaryotes.

In some aspects of the invention it is preferred that the promoter element in the DNA construct be capable of causing sufficient expression to result in the production of an effective amount of a polypeptide in water deficit conditions. Such promoters can be identified and isolated from the regulatory region of plant genes that are over expressed in water deficit conditions. Specific water-deficit-inducible promoters for use in this invention are derived from the 5' regulatory region of genes identified as a heat shock protein 17.5 gene (*HSP17.5)*, an HVA22 gene *(HVA22)*, and a cinnamic acid 4-hydroxylase (CA4H) gene *(CA4H)* of *Zea maize.* Such water-deficit-inducible promoters are disclosed in U.S. provisional application Serial No. 60/435,987, filed December 20, 2002, incorporated herein by reference.

In other aspects of the invention, sufficient expression in plant seed tissues is desired to effect improvements in seed composition. Exemplary promoters for use for seed composition modification include promoters from seed genes such as napin (U.S. Patent 5,420,034), oleosin, zein Z27 (Russell *et al.* (1997) *Transgenic Res.* 6(2):157-166), globulin 1 (Belanger *et al* (1991) Genetics 129:863-872), glutelin 1 (Russell (1997) *supra*), and peroxiredoxin antioxidant (Per1) (Stacy *et al.* (1996) *Plant Mol Biol.* 31(6):1205-1216).

In still other aspects of the invention, preferential expression in plant green tissues is desired. Promoters of interest for such uses include those from genes such as SSU (Fischhoff *et al.* (1992) Plant Mol Biol. 20:81-93), aldolase and pyruvate orthophosphate dikinase (PPDK) (WO01/19976).

Recombinant constructs prepared in accordance with the invention will also generally include a 3' untranlated DNA region that typically contains a polyadenylation sequence following the polynucleotide coding region. Examples of useful 3' UTRs include those from the nopaline synthase gene of *Agrobacterium tumefaciens* (*nos*), a gene encoding the small subunit of a ribulose-1,5-bisphosphate carboxylase-oxygenase (rbcS), and the T7 transcript of *Agrobacterium tumefaciens*.

Constructs and vectors may also include a transit peptide for targeting of a gene target to a plant organelle, particularly to a chloroplast, leucoplast or other plastid organelle. For descriptions of the use of chloroplast transit peptides see U.S. Patent 5, 188,642 and U.S. Patent No. 5,728,925, incorporated herein by reference. For description of the transit peptide region of an Arabidopsis EPSPS gene useful in the present invention, see Klee, H.J. *et al* (MGG (1987) 210:437-442).

The present invention also encompasses transgenic plants with stacked engineered traits, e.g. a crop having an enhanced phenotype resulting from expression of a recombinant polynucleotide provided herein, in combination with herbicide and/or pest resistance traits. For example, genes of the current invention can be stacked with other traits of agronomic interest, such as a trait providing herbicide resistance, for example a RoundUp Ready trait, or insect resistance, such as using a gene from *Bacillus thuringensis* to provide resistance against lepidopteran, coliopteran, homopteran, hemiopteran, and other insects. Herbicides for which resistance is useful in a plant include glyphosate herbicides, phosphinothricin herbicides, oxynil herbicides, imidazolinone herbicides, dinitroaniline herbicides, pyridine herbicides, sulfonylurea herbicides, bialaphos herbicides, sulfonamide herbicides and gluphosinate herbicides. To illustrate the that production of transgenic plants with herbicide resistance is a capability of those of ordinary skill in the art reference is made to U.S. patent application publications 2003/0106096A1 and 2002/0112260A1 and U.S. Patents 5,034,322; 5,776,760, 6,107,549 and 6,376,754, all of which are incorporated herein by reference. To illustrate that the production of transgenic plants with pest resistance is a capability of those of ordinary skill in the art reference is made to U.S. Patents 5,250,515 and 5,880,275 which disclose plants expressing an endotoxin of *Bacillus thuringiensis* bacteria, to U.S. Patent 6,506,599 which discloses control of invertebrates which feed on transgenic plants which express dsRNA for suppressing a target gene in the invertebrate, to U.S. Patent 5,986,175 which discloses the control of viral pests by

transgenic plants which express viral replicase, and to U.S. Patent Application Publication 2003/0150017 A1 which discloses control of pests by a transgenic plant which express a dsRNA targeted to suppressing a gene in the pest, all of which are incorporated herein by reference.

Plant Transformation Constructs and Methods

Constructs used for transforming plant cells will comprise the recombinant polynucleotide that one desires to introduce as well as various other elements as described above. It is also contemplated that one may employ multiple genes for expression of multiple polynucleotides for crop improvement provided herein or for expression of a polynucleotide provided herein and one or more other desirable genes on either the same or different vectors for transformation. In the latter case, the different vectors may be delivered concurrently to recipient cells if co-transformation into a single chromosomal location is desired. Numerous methods for transforming plant cells with recombinant DNA are known in the art and may be used in the present invention. Two commonly used methods for plant transformation are *Agrobacterium*-mediated transformation and microprojectile bombardment. Microprojectile bombardment methods are illustrated in U.S. Patents 5,015,580; 5,550,318; 5,538,880; 5,914,451; 6,160,208; 6,399,861 and 6,403,865 and *Agrobacterium*-mediated transformation is described in U.S. Patents 5,635,055; 5,824,877; 5,591,616; 5,981,840 and 6,384,301, all of which are incorporated herein by reference. For *Agrobacterium tumefaciens* based plant transformation system, additional elements present on transformation constructs will include T-DNA left and right border sequences to facilitate incorporation of the recombinant polynucleotide into the plant genome.

In general it is preferred to introduce heterologous DNA randomly, i.e. at a non-specific location, in the genome of a target plant line. In special cases it may be useful to target heterologous DNA insertion in order to achieve site-specific integration, e.g. to replace an existing gene in the genome, to use an existing promoter in the plant genome, or to insert a recombinant polynucleotide at a predetermined site known to be active for gene expression. Several site specific recombination systems exist which are known to function implants include cre-lox as disclosed in U.S. Patent 4,959,317 and FLP-FRT as disclosed in U.S. Patent 5,527,695, both incorporated herein by reference.

Transformation methods of this invention are preferably practiced in tissue culture on media and in a controlled environment. "Media" refers to the numerous nutrient mixtures that are used to grow cells *in vitro*, that is, outside of the intact living organism. Recipient cell targets include, but are not limited to, meristem cells, callus, immature embryos and gametic cells such as microspores, pollen, sperm and egg cells. It is contemplated that any cell from which a fertile plant may be regenerated is useful as a recipient cell. Callus may be initiated from tissue sources including, but not limited to, immature embryos, seedling apical meristems, microspores and the like. Cells capable of proliferating as callus are also recipient cells for genetic transformation. Practical transformation methods and materials for making transgenic plants of this invention, e.g. various media and recipient target cells, transformation of immature embryos and subsequent regeneration of fertile transgenic plants are disclosed in U.S. Patents 6,194,636 and 6,232,526 and U.S. patent application Serial No. 09/757,089, which are incorporated herein by reference.

In practice DNA is introduced into only a small percentage of target cells in any one experiment. Marker genes are used to provide an efficient system for identification of those cells that are stably transformed by receiving and integrating a transgenic DNA construct into their genomes. Preferred marker genes provide selective markers that confer resistance to a selective agent, such as an antibiotic or herbicide. Potentially transformed cells are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene has been integrated and expressed at sufficient levels to permit cell survival. Cells may be tested further to confirm stable integration of the exogenous DNA. Useful selective marker genes include those conferring resistance to antibiotics such as kanamycin (*nptII*), hygromycin B (*aph IV*) and gentamycin (*aac3* and *aac*C4) or resistance to herbicides such as glufosinate (*bar* or *pat*) and glyphosate (EPSPS). Examples of such selectable are illustrated in U.S. Patents 5,550,318; 5,633,435; 5,780,708 and 6,118,047, all of which are incorporated herein by reference. Screenable markers which provide an ability to visually identify transformants can also be employed, *e.g.*, a gene expressing a colored or fluorescent protein such as a luciferase or green fluorescent protein (GFP) or a gene expressing a *beta*-glucuronidase or *uid*A gene (GUS) for which various chromogenic substrates are known. It is also contemplated that combinations of screenable and selectable markers will be useful for identification of transformed cells. See PCT publication WO 99/61129 which discloses use of a

gene fusion between a selectable marker gene and a screenable marker gene, e.g. an NPTII gene and a GFP gene.

Cells that survive exposure to the selective agent, or cells that have been scored positive in a screening assay, may be cultured in regeneration media and allowed to mature into plants. Developing plantlets can be transferred to soil less plant growth mix, and hardened off, *e.g.*, in an environmentally controlled chamber at about 85% relative humidity, 600 ppm CO_2, and 25-250 microeinsteins $m^{-2} s^{-1}$ of light, prior to transfer to a greenhouse or growth chamber for maturation. Plants are preferably matured either in a growth chamber or greenhouse. Plants are regenerated from about 6 wk to 10 months after a transformant is identified, depending on the initial tissue. During regeneration, cells are grown to plants on solid media at about 19 to 28°C. After regenerating plants have reached the stage of shoot and root development, they may be transferred to a greenhouse for further growth and testing. Plants may be pollinated using conventional plant breeding methods known to those of skill in the art and seed produced.

Progeny may be recovered from transformed plants and tested for expression of the exogenous recombinant polynucleotide. Useful assays include, for example, "molecular biological" assays, such as Southern and Northern blotting and PCR; "biochemical" assays, such as detecting the presence of RNA, e.g. double stranded RNA, or a protein product, *e.g.*, by immunological means (ELISAs and Western blots) or by enzymatic function; plant part assays, such as leaf or root assays; and also, by analyzing the phenotype of the whole regenerated plant.

The present invention will be further illustrated by means of the following examples provided for illustration purposes only and in no way intended to limit the scope of the invention.

EXAMPLES

Example 1 Constructs for Maize Transformation

A GATEWAY™ Destination (Invitrogen Life Technologies, Carlsbad, CA) plant expression vector, pMON65154, was constructed for use in preparation of constructs comprising recombinant polynucleotides for corn transformation. The elements of the expression vector are summarized in Table 5 below. Generally, pMON65154 comprises a selectable marker expression cassette comprising a Cauliflower Mosaic Virus 35S promoter operably linked to a gene encoding neomycin phosphotransferase II (*npt*II). The 3' region of the selectable marker expression cassette comprises the 3' region of the *Agrobacterium tumefaciense* nopaline

synthase gene (*nos*) followed 3' by the 3' region of the potato proteinase inhibitor II (*pin*II) gene. The plasmid pMON 65154 further comprises a plant expression cassette into which a gene of interest may be inserted using GATEWAY™ cloning methods. The GATEWAY™ cloning cassette is flanked 5' by a rice actin 1 promoter, exon and intron and flanked 3' by the 3' region of the potato *pin*II gene. Using GATEWAY™ methods, the cloning cassette may be replaced with a gene of interest. The vector pMON65154, and derivatives thereof comprising a gene of interest, are particularly useful in methods of plant transformation via direct DNA delivery, such as microprojectile bombardment.

Table 5 Elements of Plasmid pMON65154

FUNCTION	ELEMENT	REFERENCE
Plant gene of interest expression cassette	Rice actin 1 promoter	U.S. Patent 5,641,876
	Rice actin 1 exon 1, intron 1 enhancer	U.S. Patent 5,641,876
Gene of interest insertion site	*Att*R1	GATEWAY™Cloning Technology Instruction Manual
	CmR gene	GATEWAY™Cloning Technology Instruction Manual
	*ccd*A, *ccd*B genes	GATEWAY™Cloning Technology Instruction Manual
	*att*R2	GATEWAY™Cloning Technology Instruction Manual
Plant gene of interest expression cassette	Potato pinII 3' region	An *et al.* (1989) Plant Cell 1:115-122
Plant selectable marker expression cassette	CaMV 35S promoter	U.S. Patent 5,858,742
	nptII selectable marker	U.S. Patent 5,858,742
	nos 3' region	U.S. Patent 5,858,742
	PinII 3' region	An *et al.* (1989) Plant Cell 1:115-122
Maintenance in *E. coli*	*ColE1* origin of replication	
	F1 origin of replication	
	Bla ampicillin resistance	

A similar plasmid vector, pMON72472, is constructed for use in *Agrobacterium* mediated methods of plant transformation. pMON72472 comprises the gene of interest plant expression cassette, GATEWAY™ cloning, and plant selectable marker expression cassettes present in pMON65154. In addition, left and right T-DNA border sequences from *Agrobacterium* are added to the plasmid (Zambryski *et al.* (1982)). The right border sequence is

located 5' to the rice actin 1 promoter and the left border sequence is located 3' to the *pin*II 3' sequence situated 3' to the *npt*II gene. Furthermore, pMON72472 comprises a plasmid backbone to facilitate replication of the plasmid in both *E. coli* and *Agrobacterium tumefaciens*. The backbone has an *ori*V wide host range origin of DNA replication functional in *Agrobacterium*, a pBR322 origin of replication functional in *E.coli*, and a spectinomycin/stretptomycin resistance gene for selection in both *E. coli* and *Agrobacterium*.

Vectors similar to those described above may be constructed for use in *Agrobacterium* or microprojectile bombardment maize transformation systems where the rice actin 1 promoter in the plant expression cassette portion is replaced with other desirable promoters including, but not limited to a maize globulin 1 promoter, a maize oleosin promoter, a glutelin 1 promoter, an aldolase promoter, a zein Z27 promoter, a pyruvate orthophosphate dikinase (PPDK) promoter, a a soybean 7S alpha promoter, a peroxiredoxin antioxidant (Perl) promoter and a CaMV 35S promoter. Protein coding segments are amplified by PCR prior to insertion into vectors such as described above. Primers for PCR amplification can be designed at or near the start and stop codons of the coding sequence, in order to eliminate most of the 5' and 3' untranslated regions. For GATEWAY cloning methods, PCR products are tailed with *att*B1 and *att*B2 sequences, purified then recombined into a destination vectors to produce an expression vector for use in transformation.

Exemplary constructs for transformation of maize to produce plants having enhanced phenotypes are provided in Table 6 below. Column headings in Table 6 refer to the following information:

"SEQ ID NO" refers to a particular nucleic acid sequence in the Sequence Listing which defines a polynucleotide used in a recombinant polynucleotide of this invention.

"PHE ID" refers to an arbitrary number used to identify a particular recombinant polynucleotide corresponding to the translated protein encoded by the polynucleotide.

"NOM ID" refers to a particular construct comprising a polynucleotide of this invention.

"GENE NAME" refers to a common name for the recombinant polynucleotide.

"PROMOTER" provides the name of the promoter region driving expression of the polynucleotide

"TARGET" indicates if a chloroplast transit peptide is employed in the construct

"pMON" refers to an arbitrary number used to designate a particular recombinant DNA construct. Constructs are prophetic where no pMON is provided.

TABLE 6 Maize Transformation Constructs

5

SEQ ID NO	Phe ID	Nom ID	Gene Name	Promoter	Target	pMON
1	PHE0000001	1	maize cellulose synthase	rice actin		-
2	PHE0000006	6	Arabidopsis RAV2/G9	rice actin		PMON68861
3	PHE0000007	7	rice G9-like 1	rice actin		-
4	PHE0000008	8/4361	rice G9-like 2	rice actin		PMON80526
5	PHE0000010	13	rice G975	rice actin		PMON67800
6	PHE0000278	14	corn G975	rice actin		PMON68886
7	PHE0000011	17	corn Glossy15	rice actin		-
8	PHE0000012	165	corn aquaporin RS81	rice actin		PMON67808
8	PHE0000012	166	antisense corn aquaporin RS81	rice actin		PMON67806
9	PHE0000014	22	rice cycD2	rice actin		PMON80471
10	PHE0000215	24	invW	rice actin		-
11	PHE0000015	25	rice GCR1	rice actin		PMON80255
12	PHE0000016	103	corn Knotted1	rice actin		PMON67750
13	PHE0000018	28	corn AAA-ATPase 2	rice actin		-
14	PHE0000019	29	rice AOX1b	rice actin		PMON80879
15	PHE0000020	30	Emericella nidulans alxA	rice actin		PMON81241
16	PHE0000022	34	corn AAP6-like	rice actin		PMON67826
17	PHE0000024	41	corn unknown protein	rice actin		PMON68354
18	PHE0000025	44	corn GRF1-like protein	rice actin		PMON68396
19	PHE0000026	45	rice GRF1	rice actin		-
20	PHE0000227	46	soy omega-3 fatty acid desaturase	rice actin		PMON68376
21	PHE0000258	47	AtFAD7	rice actin		PMON68371
22	PHE0000259	48	AtFAD8	rice actin		PMON74404
23	PHE0000049	52	rice phyA with corn phyC intron 1	rice actin		PMON80912
24	PHE0000027	53/4386	sorghum phyA with corn phyC intron 1	rice actin		PMON80920
25	PHE0000028	54	rice phyB with corn phyC intron 1	rice actin		-
26	PHE0000029	55	sorghum phyB with corn phyC intron 1	rice actin		-
27	PHE0000030	56	rice phyC with corn phyC intron 1	rice actin		-
28	PHE0000031	57	sorghum phyC with corn phyC intron 1	rice actin		-

SEQ ID NO	Phe ID	Nom ID	Gene Name	Promoter	Target	pMON
29	PHE0000032	58	rice PF1	rice actin		PMON83627
30	PHE0000033	4152	rice GT2	PPDK		-
31	PHE0000034	60	Synechocystis biliverdin reductase	rice actin		PMON67805
32	PHE0000038	65	corn cycD2.1	rice actin		PMON68383
33	PHE0000039	67	corn nph1	rice actin		PMON67807
34	PHE0000040	71	corn hemoglobin 1	rice actin		PMON67801
34	PHE0000040	74	corn hemoglobin 1	rice actin	chloroplast	PMON77889
35	PHE0000043	80	rice cyclin 2	rice actin		PMON80322
36	PHE0000044	81/4424	rice cycC	rice actin		PMON80482
37	PHE0000045	82	rice cycB2	rice actin		PMON81293
38	PHE0000046	83/4425	rice cycA1	rice actin		PMON78247
39	PHE0000047	84	rice cycB5	rice actin		-
40	PHE0000244	89	corn SVP-like	rice actin		PMON68372
41	PHE0000245	90	corn SVP-like	rice actin		PMON68373
42	PHE0000246	91	soy SVP-like	rice actin		PMON68374
43	PHE0000247	92	soy jointless-like	rice actin		PMON68375
44	PHE0000106	114/4427	corn cycA1	rice actin		PMON69457
45	PHE0000050	115	corn cycA2	rice actin		-
46	PHE0000051	116	corn cycB2	rice actin		PMON68859
47	PHE0000052	117	corn cycB5	rice actin		PMON67813
48	PHE0000382	118	LIB3279-180-C9_FLI - maize cyclin III	rice actin		PMON74401
49	PHE0000053	119	corn cycB4	rice actin		-
50	PHE0000054	120/4369	corn cycD3.2	rice actin		PMON81815
51	PHE0000055	121	corn cycDx.1	rice actin		PMON68355
52	PHE0000056	122	corn cycD1.1	rice actin		PMON68364
53	PHE0000057	124	corn mt NDK - LIB189022Q1E1E9	rice actin		PMON68350
54	PHE0000058	125	corn cp NDK - 700479629	rice actin		PMON68351
55	PHE0000059	126	corn NDK - LIB3597020Q1K6C3	rice actin		PMON68370
56	PHE0000060	127	corn NDK - 700241377	rice actin		PMON68356
57	PHE0000062	130	sRAD54 - with NLS	rice actin		-
58	PHE0000063	132	T4 endonuclease VII (gp49) - with NLS	rice actin		-
59	PHE0000064	137	corn NDPK - fC-zmemLIB3957015Q1K6H6	rice actin		PMON67804
60	PHE0000065	139/4405	TOR1	rice actin		-
61	PHE0000292	142	corn eIF-5A	rice actin		PMON68888
62	PHE0000067	143	yeast eIF-5A	rice actin		PMON67816
63	PHE0000068	144	yeast deoxyhypusine synthase	rice actin		PMON67824
64	PHE0000069	147	yeast L5	rice actin		PMON67821

SEQ ID NO	Phe ID	Nom ID	Gene Name	Promoter	Target	pMON
65	PHE0000070	149	yeast ornithine decarboxylase	rice actin		PMON67825
66	PHE0000071	151	rice exportin 4-like	rice actin		-
67	PHE0000072	152	yeast S-adenosylmethionine decarboxylase	rice actin		PMON67828
68	PHE0000073	153	corn S-adenosylmethionine decarboxylase 1	rice actin		PMON68357
69	PHE0000074	154	corn S-adenosylmethionine decarboxylase 2	rice actin		PMON68352
70	PHE0000075	155	antisense retinoblastoma-related protein 1	rice actin		-
71	PHE0000076	156	C1 protein	rice actin		PMON68851
72	PHE0000077	157	yeast flavohemoglobin - mitochondrial	rice actin		PMON67827
72	PHE0000077	158	yeast flavohemoglobin - mitochondrial	rice actin	chloroplast	PMON77890
72	PHE0000077	159	yeast flavohemoglobin - mitochondrial	rice actin		PMON75301
73	PHE0000009	164	Arabidopsis G975	rice actin		PMON67803
74	PHE0000079	169	CUT1	rice actin		PMON67752
75	PHE0000082	172	corn cycB3	rice actin		-
76	PHE0000083	173	PDR5	rice actin		PMON81229
77	PHE0000084	174	rice cyclin H	rice actin		-
78	PHE0000085	175	rice cdc2+/CDC28-related protein kinase	rice actin		PMON80475
79	PHE0000086	176	Cdk-activating kinase 1	rice actin		PMON67812
80	PHE0000089	179/4360	CHL1	rice actin		PMON80273
81	PHE0000090	180/4387	NTR1	rice actin		PMON80335
82	PHE0000091	181	Zm SET domain 2	rice actin		PMON68358
83	PHE0000092	182	Zm SET domain 1	rice actin		PMON68359
84	PHE0000095	185	HSF1	rice actin		PMON80915
85	PHE0000096	186	Zm HSP101	rice actin		-
86	PHE0000098	188	E. coli clpB	rice actin		PMON73168
87	PHE0000099	189	Synechocystis clpB	rice actin		PMON80517
88	PHE0000100	190	Xylella clpB	rice actin		PMON80917
89	PHE0000101	191/4352	corn cycD3.1	rice actin		PMON81811
90	PHE0000102	192	AnFPPS (farnesyl-pyrophosphate synthetase)	rice actin		PMON67815
91	PHE0000103	193	OsFPPS	rice actin		PMON83631
92	PHE0000104	194	700331819_FLI - corn FPPS 2	rice actin		PMON68608
93	PHE0000105	195/4426	corn cycD1.2	rice actin		PMON80329
94	PHE0000107	197/4428	corn cycD1.3	rice actin		PMON81259
95	PHE0000108	198	ASH1	rice actin		PMON67849

SEQ ID NO	Phe ID	Nom ID	Gene Name	Promoter	Target	pMON
96	PHE0000109	199	rice ASH1-like1	rice actin		-
97	PHE0000110	200	rice MtN2-like	rice actin		PMON80473
98	PHE0000111	201	PAS domain kinase	rice actin		-
99	PHE0000114	204	Su(var) 3-9-like	rice actin		PMON68361
100	PHE0000115	205	Receiver domain (RR3-like) 7	rice actin		PMON68362
101	PHE0000116	206	Receiver domain (ARR2-like) 1	rice actin		PMON68367
102	PHE0000117	207	Receiver domain (TOC1-like) 2	rice actin		PMON68368
103	PHE0000118	208	Receiver domain (TOC1-like) 3	rice actin		PMON67811
104	PHE0000119	209	Receiver domain (ARR2-like) 4	rice actin		PMON68363
105	PHE0000120	210	Receiver domain (RR11-like) 5	rice actin		PMON68853
106	PHE0000121	211	Receiver domain (RR3-like) 6	rice actin		PMON68854
107	PHE0000122	212	Receiver domain (RR3-like) 8	rice actin		PMON74402
108	PHE0000123	213	Receiver domain 9	rice actin		PMON68855
109	PHE0000124	214	ZmRR2	rice actin		PMON68856
110	PHE0000125	215	Receiver domain (TOC1-like) 10	rice actin		PMON68369
111	PHE0000126	216	corn HY5-like	rice actin		PMON69458
112	PHE0000127	217	scarecrow 1 (PAT1-like)	rice actin		PMON68887
113	PHE0000128	218	scarecrow 2	rice actin		-
114	PHE0000133	223	G protein b subunit	rice actin		PMON68860
115	PHE0000152	242	14-3-3-like protein 2	rice actin		PMON77899
116	PHE0000153	243	14-3-3-like protein D	rice actin		PMON67817
117	PHE0000154	244	14-3-3 protein 1	rice actin		PMON67818
118	PHE0000155	245	Rice FAP1-like protein	rice actin		-
119	PHE0000156	246	rice TAP42-like	rice actin		-
120	PHE0000158	248	BMH1	rice actin		PMON73169
121	PHE0000159	250	rice chloroplastic fructose-1,6-bisphosphatase	rice actin		PMON83640
122	PHE0000160	251	E. coli fructose-1,6-bisphosphatase	rice actin	chloroplast	PMON75485
123	PHE0000161	252	Synechocystis fructose-1,6-bisphosphatase F-I	rice actin	chloroplast	PMON82231
124	PHE0000162	3383	Synechocystis fructose-1,6-bisphosphatase F-II	Glutellin1 3.1 kb		-
124	PHE0000162	253	Synechocystis fructose-1,6-bisphosphatase F-II	rice actin	chloroplast	PMON75488
125	PHE0000164	255	Yeast RPT5	rice actin		PMON73170
126	PHE0000165	257	Yeast RRP5	rice actin		PMON81210

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127	PHE0000166	258	Rice CBP-like gene	rice actin		-
128	PHE0000167	259	rice BAB09754	rice actin		PMON80340
129	PHE0000168	260	LIB3061-001-H7_FLI	rice actin		PMON68857
130	PHE0000169	262	maize p23	rice actin		-
131	PHE0000170	263	maize cyclophilin	rice actin		PMON81258
132	PHE0000172	265	yeast SIT1	rice actin		PMON81206
133	PHE0000173	266	yeast CNS1	rice actin		PMON73171
134	PHE0000176	269	RNAse S	rice actin		PMON68388
135	PHE0000177	270	maize ecto-apyrase	rice actin		PMON68881
136	PHE0000178	271	PHO5	rice actin		PMON73166
137	PHE0000179	272	high affinity phosphate translocator	rice actin		PMON69467
138	PHE0000180	273	high affinity phosphate translocator	rice actin		PMON83753
139	PHE0000181	274	Xylella citrate synthase	rice actin		PMON76326
140	PHE0000182	275	E. coli citrate synthase	rice actin		PMON74420
141	PHE0000183	276	rice citrate synthase	rice actin		PMON80258
142	PHE0000184	277/4429	citrate synthase	rice actin		PMON81278
143	PHE0000185	278	citrate synthase	rice actin		PMON69468
144	PHE0000186	279	maize ferritin 2	rice actin		PMON69460
145	PHE0000187	280	maize ferritin 1	rice actin		PMON81261
146	PHE0000188	281	E. coli cytoplasmic ferritin	rice actin		PMON73167
147	PHE0000190	283	corn LEA3	rice actin		-
148	PHE0000192	285	soy HSF	rice actin		PMON68394
149	PHE0000193	286	soy HSF	rice actin		PMON68889
150	PHE0000204	297	deoxyhypusine synthase	rice actin		-
151	PHE0000219	308	thylakoid carbonic anhydrase, cah3	rice actin		PMON68865
152	PHE0000216	309	thylakoid carbonic anhydrase, ecaA	rice actin		PMON81823
153	PHE0000217	310	Chlamydomonas reinhardtii envelope protein LIP-36G1	rice actin		-
154	PHE0000218	311/4431	psbO transit peptide::Synechococcus sp. PCC 7942 ictB	rice actin	chloroplast lumen	-
155	PHE0000220	313	corn RNase PH	rice actin		PMON74434
156	PHE0000221	314	SKI2	rice actin		-
157	PHE0000222	315	SKI3	rice actin		PMON80320
158	PHE0000223	316	SKI4	rice actin		PMON69478
159	PHE0000224	317	SKI6	rice actin		PMON80278
160	PHE0000225	318	SKI7	rice actin		-
161	PHE0000226	319	rice SKI7-like	rice actin		-
162	PHE0000228	321	Synechocystis cobA w cp transit	rice actin	chloroplast	-

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			peptide			
163	PHE0000229	324	Xylella tetrapyrrole methylase with transit peptide	rice actin	chloroplast	PMON77900
164	PHE0000230	325	maize uroporphyrinogen III methyltransferase	rice actin		-
165	PHE0000231	326	nucellin-like protein	rice actin		PMON72498
166	PHE0000232	327	nucellin-like protein	rice actin		PMON68895
167	PHE0000233	328	nucellin-like protein	rice actin		PMON82671
168	PHE0000234	329	soy LEA protein	rice actin		PMON73159
169	PHE0000235	330	dehydrin-like protein	rice actin		PMON73161
170	PHE0000237	332	dehydrin 3	rice actin		PMON68891
171	PHE0000238	333	probable lipase	rice actin		PMON69466
172	PHE0000239	334	yeast GRE1	rice actin		PMON72466
173	PHE0000240	335	yeast STF2	rice actin		PMON72468
174	PHE0000241	336	yeast SIP18	rice actin		-
175	PHE0000242	337	yeast YBM6	rice actin		PMON72470
176	PHE0000243	338	yeast HSP12	rice actin		PMON72467
177	PHE0000249	340	corn allene oxide synthase	rice actin		PMON74422
178	PHE0000250	341	corn COI1-like	rice actin		PMON82194
179	PHE0000251	342	corn TIR1-like	rice actin		-
180	PHE0000252	343	corn COI1-like	rice actin		PMON74407
181	PHE0000253	344	COI1-like	rice actin		-
182	PHE0000254	345	F-box protein	rice actin		PMON73172
183	PHE0000255	346	F-box protein	rice actin		PMON72459
184	PHE0000256	347	corn 1-aminocyclopropane-1-carboxylate oxidase	rice actin		PMON75302
185	PHE0000257	348	rice 1-aminocyclopropane-1 carboxylate synthase	rice actin		PMON80260
186	PHE0000260	349	S52650 - Synechocystis desB	rice actin	chloroplast	PMON75487
187	PHE0000261	350	yeast glutamate decarboxylase	rice actin		PMON80276
188	PHE0000262	352	cytochrome P450-like protein	rice actin		PMON68892
189	PHE0000263	353	cytochrome P450	rice actin		PMON74412
190	PHE0000264	354	cytochrome P450-like	rice actin		PMON68866
191	PHE0000265	355	CYP90 protein	rice actin		PMON69469
192	PHE0000266	356	cytochrome P450 DWARF3	rice actin		PMON69470
193	PHE0000267	357	cytochrome P450	rice actin		PMON68867
194	PHE0000268	358	rice receptor protein kinase	rice actin		-
195	PHE0000269	359	soy E2F-like	rice actin		-

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196	PHE0000270	360/4432	nuclear matrix constituent protein	rice actin		PMON80316
197	PHE0000271	361/4433	OsE2F1	rice actin		-
198	PHE0000272	362	corn GCR1	rice actin		-
199	PHE0000273	363	soy mlo-like	rice actin		PMON74423
200	PHE0000274	364	soy mlo-like	rice actin		-
201	PHE0000275	365	rice G alpha 1	rice actin		PMON80259
202	PHE0000276	366	soy G-gamma subunit	rice actin		PMON68868
203	PHE0000277	368	wheat G28-like	rice actin		PMON68890
204	PHE0000279	369	sorghum proline permease	rice actin		PMON68896
205	PHE0000280	370	rice AA transporter	rice actin		PMON72451
206	PHE0000282	372	SET-domain protein-like	rice actin		-
207	PHE0000283	373	scarecrow 6	rice actin		PMON69472
208	PHE0000284	374	menage a trois-like	rice actin		PMON72453
209	PHE0000286	376	oryzacystatin	rice actin		PMON72454
210	PHE0000287	377	Similar to cysteine proteinase inhibitor	rice actin		PMON68898
211	PHE0000288	378	cysteine proteinase inhibitor	rice actin		-
212	PHE0000289	379	Zm-GRF1 (GA responsive factor)	rice actin		-
213	PHE0000290	380	ZmSE001-like	rice actin		-
214	PHE0000291	381	deoxyhypusine synthase	rice actin		PMON72455
215	PHE0000293	382/4368	gibberellin response modulator	rice actin		PMON75972
216	PHE0000294	383	scarecrow-like protein	rice actin		PMON68897
217	PHE0000295	384	ubiquitin-conjugating enzyme-like protein	rice actin		PMON68894
218	PHE0000296	385	unknown protein recognized by PF01169	rice actin		PMON68893
219	PHE0000297	386	26S protease regulatory subunit 6A homolog	rice actin		PMON68899
220	PHE0000298	387	rice p23 co-chaperone	rice actin		PMON68874
221	PHE0000299	388	corn p23 co-chaperone	rice actin		PMON68875
222	PHE0000300	389	rice p23 co-chaperone	rice actin		PMON68876
223	PHE0000301	390	corn p23 co-chaperone	rice actin		PMON68877
224	PHE0000302	391	putative purple acid phosphatase precursor	rice actin		PMON68878
225	PHE0000303	392	acid phosphatase type 5	rice actin		PMON68879
226	PHE0000304	393	aleurone ribonuclease	rice actin		PMON68873
227	PHE0000305	394	putative ribonuclease	rice actin		PMON68880
228	PHE0000306	395	S-like RNase	rice actin		PMON68882
229	PHE0000307	396	ribonuclease	rice actin		PMON68883
230	PHE0000308	397	helix-loop-helix protein (PIF3-	rice actin		PMON68884

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			like)			
231	PHE0000309	398	SKI4-like protein	rice actin		PMON68885
232	PHE0000310	399	putative 3 exoribonuclease	rice actin		PMON68377
233	PHE0000311	400	GF14-c protein	rice actin		PMON72458
234	PHE0000312	401	14-3-3-like protein	rice actin		PMON72456
235	PHE0000313	402	rice eIF-(iso)4F	rice actin		PMON68378
236	PHE0000314	403	rice eIF-4F	rice actin		PMON68379
237	PHE0000315	404	sorghum eIF-(iso)4F	rice actin		PMON68381
238	PHE0000316	405	sorghum eIF-4F	rice actin		PMON68382
239	PHE0000317	406	rice FIP37-like	rice actin		PMON68380
240	PHE0000318	407	scarecrow 17	rice actin		PMON81878
241	PHE0000322	411	maize catalase-1	rice actin		PMON74403
242	PHE0000323	412	maize catalase-3	rice actin		PMON68400
243	PHE0000324	413	ascorbate peroxidase	rice actin		PMON73162
244	PHE0000325	414	corn GDI	rice actin		PMON68384
245	PHE0000326	415	soy GDI	rice actin		PMON72463
246	PHE0000327	416	corn rho GDI	rice actin		PMON69481
247	PHE0000328	417	basic blue copper protein	rice actin		PMON74416
248	PHE0000329	418	plantacyanin	rice actin		PMON80945
249	PHE0000330	419	basic blue copper protein	rice actin		PMON73164
250	PHE0000331	420	Similar to blue copper protein precursor	rice actin		-
251	PHE0000332	421	lamin	rice actin		PMON68385
252	PHE0000333	422	fC-zmfl700551169a-allyl alcohol dehydrogenase	rice actin		PMON75470
253	PHE0000334	423	allyl alcohol dehydrogenase	rice actin		PMON68395
254	PHE0000335	424	allyl alcohol dehydrogenase	rice actin		PMON74413
255	PHE0000336	425	qui oxidoreductase	rice actin		PMON74414
256	PHE0000337	426	E. nidulans cysA - AF029885	rice actin		-
257	PHE0000338	427	BAA18167 - Synechocystis cysE	rice actin		PMON68628
258	PHE0000339	428	Synechocystis thiol-specific antioxidant protein - BAA10136	rice actin		PMON68627
258	PHE0000339	429	Synechocystis thiol-specific antioxidant protein - BAA10136	rice actin	chloroplast	PMON75490
259	PHE0000340	430	yeast TSA2 - NP_010741	rice actin		PMON68629
260	PHE0000341	431	yeast mTPx - Z35825	rice actin		PMON68397
261	PHE0000343	433	yeast TPx III - NP_013210	rice actin		PMON80506

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262	PHE0000345	435	soy putative 2-cys peroxiredoxin	rice actin		PMON74411
263	PHE0000346	436	soy peroxiredoxin	rice actin		PMON73165
264	PHE0000347	437	heat shock protein 26, plastid-localized	rice actin		PMON68386
265	PHE0000349	439	heat shock protein	rice actin		PMON68389
266	PHE0000350	440	low molecular weight heat shock protein	rice actin		PMON74410
267	PHE0000351	441	18kDa heat shock protein	rice actin		-
268	PHE0000352	442	heat shock protein 16.9	rice actin		PMON74409
269	PHE0000353	443	HSP21-like protein	rice actin		PMON73160
270	PHE0000354	444	Opt1p - NP_012323	rice actin		PMON81879
271	PHE0000355	445	SVCT2-like permease	rice actin		PMON83797
272	PHE0000356	446	SVCT2-like permease	rice actin		PMON72464
273	PHE0000357	447	maize tubby-like	rice actin		PMON69474
274	PHE0000358	449	maize tubby-like	rice actin		PMON69475
275	PHE0000359	450	soy HMG CoA synthase	rice actin		PMON69476
276	PHE0000360	451	yeast HMGS - X96617	rice actin		PMON81886
277	PHE0000361	452	PAT1-like scarecrow 9	rice actin		PMON78900
278	PHE0000362	453	CDC28-related protein kinase	rice actin		PMON81840
279	PHE0000385	474	H+ transporting ATPase	rice actin		PMON75498
280	PHE0000386	475	cation-transporting ATPase	rice actin		PMON67834
281	PHE0000387	476	yeast DRS2 (ALA1-like) - L01795	rice actin		-
282	PHE0000388	477	S. pombe ALA1-like-CAA21897	rice actin		-
283	PHE0000389	478	rice ALA1-like 1 - BAA89544	rice actin		PMON80290
284	PHE0000390	479/4340	rice chloroplastic sedoheptulose-1,7-bisphosphatase-	rice actin		PMON67836
285	PHE0000391	480/4434	rice cytosolic fructose-1,6-bisphosphatase	rice actin		PMON67835
286	PHE0000392	481	Wheat sedoheptulose-1,7-bisphosphatase	rice actin		PMON76335
287	PHE0000394	483	sedoheptulose-1,7-bisphosphatase	rice actin		-
288	PHE0000395	484	soy phantastica (rough sheath 2-like)	rice actin		PMON67840
289	PHE0000396	485	soy phantastica 2 (rough sheath 2-like)	rice actin		PMON67838
290	PHE0000397	486	maize rough sheath 1	rice actin		PMON67839
291	PHE0000398	487	soy lg3-like 1	rice actin		PMON72488
292	PHE0000399	488	soy rough sheath1-like 1	rice actin		PMON72485

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293	PHE0000400	489	soy G559-like	rice actin		PMON72486
294	PHE0000401	490	soy G1635-like 1	rice actin		PMON67837
295	PHE0000402	491	rice amino acid transporter-like protein	rice actin		PMON67833
296	PHE0000403	492/4341	corn amino acid permease	rice actin		PMON67831
297	PHE0000404	493	rice proline transport protein	rice actin		PMON67832
298	PHE0000412	501	corn monosaccharide transporter 1	rice actin		PMON67843
299	PHE0000413	502	soy monosaccharide transporter 3	rice actin		PMON67844
300	PHE0000414	503	corn monosaccharide transporter 3	rice actin		PMON67845
301	PHE0000415	504	soy monosaccharide transporter 1	rice actin		PMON67846
302	PHE0000416	505	corn monosaccharide transporter 6	rice actin		PMON67847
303	PHE0000418	507	corn monosaccharide transporter 4	rice actin		PMON69497
304	PHE0000419	508	soy monosaccharide transporter 2	rice actin		PMON67848
305	PHE0000420	509	soy sucrose transporter	rice actin		PMON74415
306	PHE0000421	510	corn sucrose transporter 2	rice actin		PMON83760
307	PHE0000422	511	corn monosaccharide transporter 8	rice actin		PMON79433
308	PHE0000423	512	corn monosaccharide transporter 7	rice actin		PMON72497
309	PHE0000425	514	soy isoflavone synthase	rice actin		PMON72495
310	PHE0000426	515	soy ttg1-like 2	rice actin		PMON74408
311	PHE0000427	516	GATE5 - corn SPA1-like 1	rice actin		-
312	PHE0000428	517	corn PIF3-like	rice actin		PMON74417
313	PHE0000429	518	soy Athb-2-like 1	rice actin		PMON74418
314	PHE0000430	519	corn SUB1-like 1	rice actin		-
315	PHE0000431	520/4435	soy GH3 protein	rice actin		PMON81262
316	PHE0000432	521	corn 12-oxophytodienoate reductase 1	rice actin		PMON79441
317	PHE0000433	522	corn 12-oxo-phytodienoate reductase-like 3	rice actin		PMON74424
318	PHE0000434	523	corn 12-oxophytodienoate reductase-like 4	rice actin		PMON74419
319	PHE0000435	524	corn hydroperoxide lyase	rice actin		PMON75499
320	PHE0000436	525	rice cns1-like	rice actin		PMON79442

SEQ ID NO	Phe ID	N m ID	Gene Name	Promoter	Target	pMON
321	PHE0000437	526	corn HCH1-like 1	rice actin		PMON68630
322	PHE0000438	527	corn HOP-like 1	rice actin		PMON74433
323	PHE0000439	528	corn HOP-like 2	rice actin		PMON74425
324	PHE0000440	529	rice CHIP-like 1	rice actin		PMON72473
325	PHE0000441	530	corn CHIP-like 2	rice actin		PMON72474
326	PHE0000451	540	wheat SVP-like 1	rice actin		PMON72475
327	PHE0000452	541	corn SVP-like 3	rice actin		PMON72476
328	PHE0000453	542	corn SVP-like 5	rice actin		-
329	PHE0000454	543	fC-zmhuLIB3062-044-Q1-K1-B8	rice actin		PMON72477
330	PHE0000455	544	corn E4/E8 binding protein-like	rice actin		-
331	PHE0000469	558	yeast YKL091c - Z28091	rice actin		PMON68636
332	PHE0000470	559	corn Ssh1-like protein 1	rice actin		PMON79435
333	PHE0000471	560	corn Ssh1-like protein 3	rice actin		PMON73772
334	PHE0000472	561	corn Ssh1-like protein 4	rice actin		PMON79436
335	PHE0000473	562	soy Ssh1-like protein 2 [ssh2]	rice actin		PMON75471
336	PHE0000484	573	soy JMT-like protien 1	rice actin		PMON81287
337	PHE0000485	574	corn JMT-like protein 1	rice actin		PMON69498
338	PHE0000486	575	corn JMT-like protein 2	rice actin		PMON69496
339	PHE0000017	27	corn AAA-ATPase 1	rice actin		PMON68850
339	PHE0000017	686	corn AAA-ATPase 1	rice actin		PMON72479

Example 2 Constructs for Soybean Transformation

Constructs for use in transformation of soybean may be prepared by restriction enzyme based cloning into a common expression vector. Elements of an exemplary common expression vector are shown in Table 7 below.

TABLE 7 Elements of pMON74532

Function	Element	Reference
Agro transformation	B-ARGtu.right border	Depicker, A. et al (1982) Mol Appl Genet 1:561-573
Antibiotic resistance	CR-Ec.aadA-SPC/STR	
Repressor of primers from the ColE1 plasmid	CR-Ec.rop	
Origin of replication	OR-Ec.oriV-RK2	
Agro transformation	B-ARGtu.left border	Barker, R.F. et al (1983) Plant Mol Biol 2:335-350

Function	Element	Reference
Plant selectable marker expression cassette	Promoter with intron and 5'UTR of arabidopsis act 7 gene (AtAct7)	McDowell *et al.* (1996) Plant Physiol. 111:699-711.
	5' UTR of arabidopsis act 7 gene	
	Intron in 5'UTR of AtAct7	
	Transit peptide region of Arabidopsis EPSPS	Klee, H.J. *et al* (1987) MGG 210:437-442
	Synthetic CP4 coding region with dicot preferred codon usage	U.S. Patent 6,248,876
	A 3' UTR of the nopaline synthase gene of Agrobacterium tumefaciens Ti plasmid	U.S. Patent 5,858,742
Plant gene of interest expression cassette	Promoter for 35S RNA from CaMV containing a duplication of the -90 to -350 region (e35S)	U.S. Patent 5,322,938
	Gene of interest insertion site	
	Cotton E6 3' end	GenBank accession U30508

Vectors similar to that described above may be constructed for use in *Agrobacterium* mediated soybean transformation systems where the enhanced 35S promoter in the plant expression cassette portion is replaced with other desirable promoters including, but not limited to a napin promoter and an *Arabidopsis* SSU promoter. Protein coding segments are amplified by PCR prior to insertion into vectors such as described above. Primers for PCR amplification can be designed at or near the start and stop codons of the coding sequence, in order to eliminate most of the 5' and 3' untranslated regions.

Exemplary sense constructs for transformation of soybean to produce plants having enhanced phenotypes are provided in Table 8 below. Column headings in Table 8 refer to the following information:

"SEQ ID NO" refers to a particular nucleic acid sequence in the Sequence Listing which defines a polynucleotide used in a recombinant polynucleotide of this invention.

"PHE ID" refers to an arbitrary number used to identify a particular recombinant polynucleotide corresponding to the translated protein encoded by the polynucleotide.

"NOM ID" refers to a particular construct comprising a polynucleotide of this invention.

"GENE NAME" refers to a common name for the recombinant polynucleotide.

"PROMOTER" provides the name of the promoter region driving expression of the polynucleotide

"pMON" refers to an arbitrary number used to designate a particular recombinant DNA construct. Constructs are prophetic where no pMON is provided.

"Gene effect contributing to increased yield" describes the effect of the recombinant polynucleotide on the plant in providing yield improvement.

TABLE 8 Soybean Transformation Constructs

SEQ ID NO	Phe ID	Nom ID	Gene Name	Promoter	pMON	Gene effect contributing to increased yield
8	PHE0000012	4249	corn aquaporin RS81	e35S	pMON 83080	Increased root mass
34	PHE0000040	3968	corn hemoglobin 1	e35S	pMON 83103	Cold tolerance
53	PHE0000057	3962	corn mt NDK - LIB189022Q1E1E9	e35S	pMON 83055	Abiotic stress tolerance
62	PHE0000067	4248	yeast eIF-5A	e35S	pMON83076	Nitrogen use efficiency
123	PHE0000161	3578	Synechocystis fructose-1,6-bisphosphatase F-I	e35S	pMON 81321	Increased sucrose production/transport
204	PHE0000279	4246	sorghum proline permease	e35S	pMON 83093	Nitrogen use efficiency
234	PHE0000312	4247	14-3-3-like protein	e35S	pMON83075	Nitrogen use efficiency
236	PHE0000314	4245	rice eIF-4F	e35S	pMON83074	Nitrogen use efficiency
253	PHE0000334	4268	allyl alcohol dehydrogenase	e35S	pMON 84409	Heat tolerance

Example 3 Plant Transformation

Maize Transformation

LH59 plants are grown in the greenhouse and ears and ears harvested. when the embryos are 1.5 to 2.0 mm in length. Ears were surface sterilized by spraying or soaking the ears in 80% ethanol, followed by air drying. Immature embryos were isolated from individual kernels on surface sterilized ears. Prior to inoculation of maize cells, *Agrobacterium* cells are grown overnight at room temperature. Immature maize embryos are inoculated with *Agrobacterium* shortly after excision, and incubated at room temperature with *Agrobacterium* for 5-20 minutes.

Immature embryos are then co-cultured with *Agrobacterium* for 1 to 3 days at 23°C in the dark. Co-cultured embryos are transferred to selection media and cultured for approximately two weeks to allow embryogenic callus to develop. Embryogenic callus is transferred to culture medium containing 100 mg/L paromomycin and subcultured at about two week intervals. Transformants are recovered 6 to 8 weeks after initiation of selection.

For *Agrobacterium* mediated transformation of maize callus, immature embryos are cultured for approximately 8-21 days after excision to allow callus to develop. Callus is then incubated for about 30 minutes at room temperature with the *Agrobacterium* suspension, followed by removal of the liquid by aspiration. The callus and *Agrobacterium* are co-cultured without selection for 3-6 days followed by selection on paromomycin for approximately 6 weeks, with biweekly transfers to fresh media, and paromomycin resistant callus identified.

For transformation by microprojectile bombardment, immature maize embryos are isolated and cultured 3-4 days prior to bombardment. Prior to microprojectile bombardment, a suspension of gold particles is prepared onto which the desired DNA is precipitated. DNA is introduced into maize cells as described in U.S. Patent No. 5,015,580 using the electric discharge particle acceleration gene delivery device. For microprojectile bombardment of LH59 pre-cultured immature embryos, 35% to 45% of maximum voltage is preferably used. Following microprojectile bombardment, tissue is cultured in the dark at 27°C.

Fertile transgenic plants are produced from transformed maize cells by transfer of. transformed callus to appropriate regeneration media to initiate shoot development. Plantlets are transferred to soil when they are about 3 inches tall and have roots (about four to 6 weeks after transfer to medium). Plants are maintained for two weeks in a growth chamber at 26°C, followed by two weeks on a mist bench in a greenhouse before transplanting to 5 gallon pots for greenhouse growth. Plants are grown in the greenhouse to maturity and reciprocal pollinations made with the inbred LH59. Seed is collected from plants and used for further breeding activities.

Transformation methods and materials for making transgenic plants of this invention, e.g. various media and recipient target cells, transformation of immature embryos and subsequent regeneration of fertile transgenic plants are disclosed in U.S. Patents 6,194,636 and 6,232,526 and U.S. patent application Serial No. 09/757,089, which are incorporated herein by reference.

Soybean Transformation

For *Agrobacterium* mediated transformation, soybean seeds are germinated overnight and the meristem explants excised. The meristems and the explants are placed in a wounding vessel. Soybean explants and induced *Agrobacterium* cells from a strain containing plasmid DNA with the gene of interest cassette and a plant selectable marker cassette are mixed no later than 14 hours from the time of initiation of seed germination and wounded using sonication. Following wounding, explants are placed in co-culture for 2-5 days at which point they are transferred to selection media for 6-8 weeks to allow selection and growth of transgenic shoots. Phenotype positive shoots are harvested approximately 6-8 weeks post bombardment and placed into selective rooting media for 2-3 weeks. Shoots producing roots are transferred to the greenhouse and potted in soil. Shoots that remain healthy on selection, but do not produce roots are transferred to non-selective rooting media for an additional two weeks. Roots from any shoots that produce roots off selection are tested for expression of the plant selectable marker before they are transferred to the greenhouse and potted in soil.

Descriptions of media useful for transformation and regeneration of soybean and a method employing microprojectile bombardment are described in US patent 5,914,451, which is incorporated herein by reference.

Example 4 Identification of Homologs

A BLAST searchable "All Protein Database" was constructed of known protein sequences using a proprietary sequence database and the National Center for Biotechnology Information (NCBI) non-redundant amino acid database (nr.aa). For each organism from which a polynucleotide sequence provided herein was obtained, an "Organism Protein Database" was constructed of known protein sequences of the organism; it is a subset of the All Protein Database based on the NCBI taxonomy ID for the organism. Nucleotide sequences of genes provided herein are identified by SEQ ID NO in Table 1. SEQ ID NOs of amino acid sequences and organism name for polypeptides encoded by the polynucleotides provided herein are shown in Table 2.

The All Protein Database was queried using polypeptide sequences provided herein as SEQ ID NO: 340 through SEQ ID NO:678 using "blastp" with E-value cutoff of 1e-8. Up to 1000 top hits were kept, and separated by organism names. For each organism other than that of

the query sequence, a list was kept for hits from the query organism itself with a more significant E-value than the best hit of the organism. The list contains likely duplicated genes of the polynucleotides provided herein, and is referred to as the Core List. Another list was kept for all the hits from each organism, sorted by E-value, and referred to as the Hit List.

The Organism Protein Database was queried using polypeptide sequences provided herein as SEQ ID NO: 340 through SEQ ID NO:678 using "blastp" with E-value cutoff of 1e-4. Up to 1000 top hits were kept. A BLAST searchable database was constructed based on these hits, and is referred to as "SubDB". SubDB was queried with each sequence in the Hit List using "blastp" with E-value cutoff of 1e-8. The hit with the best E-value was compared with the Core List from the corresponding organism. The hit is deemed a likely ortholog if it belongs to the Core List, otherwise it is deemed not a likely oththolog and there is no further search of sequences in the Hit List for the same organism. Likely orthologs from a large number of distinct organisms were identified and are reported by amino acid sequences of SEQ ID NO:679 to SEQ ID NO: 24149. These orthologs are reported in Table 2 as homologs to the 339 polypeptides provided herein. Table 3 provides the SEQ ID NO and the name of the organism in which it was identified for each homolog gene.

All publications and patent applications cited herein are incorporated by reference in their entirely to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.